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12

TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

STUDIES ON FATE OF PARATHION IN RABBITS, USING RADIOACTIVE ISOTOPE TECHNIQUES

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STUDIES on the toxicity of parathion to mammals have consisted largely in determining $L.D._{50}$ values and cholinesterase-inhibitory effects. The fate of the compound has not been clarified sufficiently, and only a few investigators have examined this important phase of the toxicology of parathion. Hazleton and Holland[1] and Gardocki and Hazleton[2] have reported traces of parathion or its degradation products in the urine of dogs following intravenous administration of toxic amounts of the compound. Dahm and associates[3] reported that parathion is not excreted in the milk of dairy cows fed parathion-wettable powder at levels of 1 or 5 ppm of the estimated dry rough intake. In a recent and similar experiment Pankaskie and associates[4] were unable to demonstrate the presence of parathion or free *p*-nitrophenol in the milk, although the average intake of parathion per cow was 167 mg. per day; nor could parathion, free *p*-nitrophenol or free *p*-aminophenol be found in jugular blood, urine, or milk when water-wettable powder was fed in capsules at increasing rates of from 1 to 32 mg. of parathion per kilogram of cow body weight per day. *p*-Aminophenol, however, occurred in the urine but represented only about 10% of the total parathion fed. Lehman, according to Gardocki and Hazleton[2] and Hazleton and Holland,[1] found that parathion when administered in toxic doses was not stored in the body. The colorimetric method of Averell and Norris[5] was used for this determination. Until the present, no one has reported on the fate of the thiophosphoric acid portion of the parathion molecule. The present paper reports on the disposition of parathion labeled with radioactive S^{35} after dermal and intravenous administration to rabbits.

From the Communicable Disease Center, Public Health Service, Federal Security Agency.

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6

EXPERIMENTAL PROCEDURE

The radioactive S^{35}-labeled parathion used throughout the work was prepared by Jensen and Pearce.[6] It had an approximate specific activity of 220 μc per millimole.

In preliminary studies of possible methods of making radioassays of fluids and tissues taken from experimental animals,[7] a micro-Carius sulfur[8] technique was investigated. While this procedure is perhaps the most accurate available, it is somewhat slow and laborious. The possibility of making radioassays directly on smears of blood, urine, and macerated or partially digested tissues was studied, and a simplified technique was developed and used throughout this study. The success of the procedure apparently depends on the fact that parathion or a sulfur-bearing part of the molecule is readily immobilized by blood and presumably by certain other biological materials. The vapor pressure of parathion is too high to permit accurate, direct counting unless this occurs.

The direct counting procedure as applied to blood consisted in spreading 0.025 ml. evenly on 22 mm. square cover glasses covering a total area of approximately 3.25 sq. cm. The smears were then dried at 30 to 35 C. under a heat lamp and were assayed for radioactivity in a "Q" gas open-window Geiger counter (Model D-46, Nuclear Instrument & Chemical Corp.). By addition of known amounts of the radioactive parathion to blood and by counting in the same manner, it was determined that a net of 10 counts per minute (cpm) was equivalent to 0.004 γ of parathion. In a similar manner, cpm calibration in terms of parathion equivalent in the case of other media was made. The data in Table 1 illustrate the assaying reproducibility in terms of cpm between duplicate samples of rabbit blood. The agreement between duplicates was deemed adequate for the present study.

TABLE 1.—*Comparison of Paired Aliquots of Rabbit Blood Assayed by Direct Count of Smears*

Sample	Counts per Minute	
	Aliquot A	Aliquot B
1	9.8	9.7
2	10.0	6.8
2	20.8	20.6
4	44.1	38.4
5	56.0	54.0
6	74.6	75.7

Urine (0.3 ml.) was evaporated to dryness in aluminum pans and assayed directly for radioactivity. Assay of feces, on the other hand, gave extremely variable results, suggesting contamination by urine, and the sampling of feces was therefore discontinued. However, uncontaminated feces recovered at autopsy did show small but discrete amounts of radioactive material.

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[6] Jensen, J. A., and Pearce, G. W.: Synthesis of Radioactive Parathion Using S^{35}, J. Am. Chem. Soc. **74**:3184, 1952.

[7] Henriques, F. C., Jr.; Kistiakowsky, G. B.; Margnetti, C., and Schneider, W. F.: Analytical Procedure for Measurement of Long-Lived Radioactive Sulfur, S^{35}, with a Lauritzen Electroscope and Comparison of Electroscope with Special Geiger Counter, Indust. & Engin. Chem., (Analyt. Ed.) **18**:349, 1946.

[8] Niederl, J. B.; Baum, H.; McCoy, J. S., and Kuck, J. A.: Microcarius Halogens and Sulfur Determination, Indust. & Engin. Chem. (Analyt. Ed.) **12**:428, 1940.

After these preliminary studies, rabbits were treated dermally with 125 mg. of the labeled parathion applied on a shaved portion of skin over the coxal bone. Blood samples were collected beginning 15 minutes following application and continuing at increasing intervals up to 18 days. Daily urine samples were also collected over the same period.

Although in some animals low levels of radioactivity were observed in the blood within 15 minutes after dosage, only trace amounts were detectable in others. The urine has always been found extremely active, and apparently the excretory system is very efficient in removing parathion, or at least the sulfur-bearing portion of the molecule, from the blood stream. In two rabbits appreciable quantities of activity were found in the blood, and the averaged data (expressed as parathion equivalent) for these animals are presented in chart 1. The average amounts found in the urine of the same animals are also given in the figure. It will be noted that it was necessary to magnify the lower portion of the ordinate scale in order to show values for both blood and urine on the same chart. The important observation to be made from these data is that the amounts likely to be found in the blood are very small and most of the activity appears in the urine.

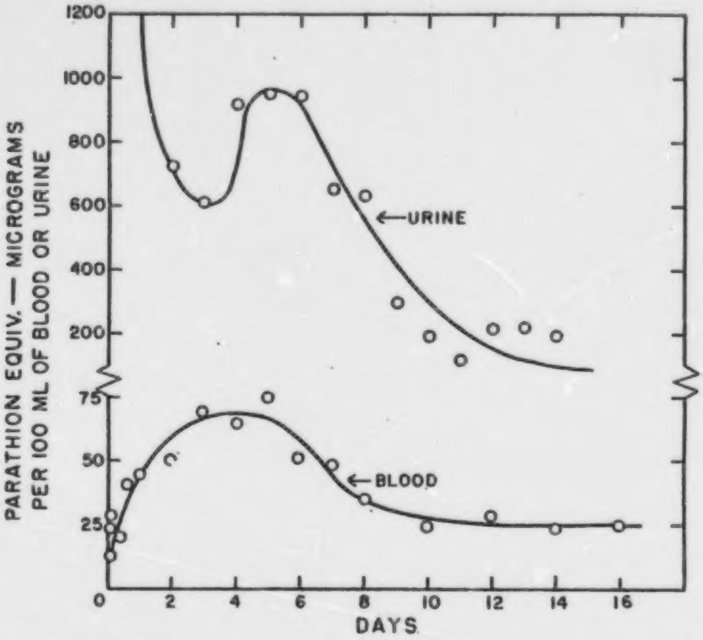

Chart 1.—Parathion equivalent in blood and urine of rabbits following dermal application.

To test further the apparent rapidity of excretion of parathion or a degradation product, a rabbit was treated intravenously with 20 mg. of parathion (6 mg. kg.) in 0.5 ml. of ethyl alcohol. The dosage was injected in the vein of one ear, and blood samples were removed from the opposite ear starting five minutes after dosing and continuing at intervals for the next five hours. The parathion equivalent found in the blood has been plotted against time in chart 2. It seems clear from this experiment that the excretory system can remove near-lethal dosages of parathion from the blood in a very short time.

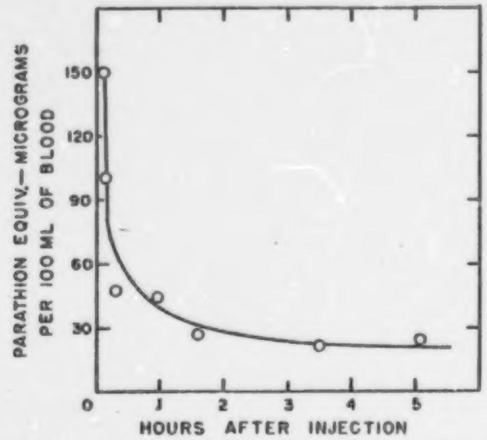

Chart 2.—Elimination of parathion after intravenous administration in rabbits.

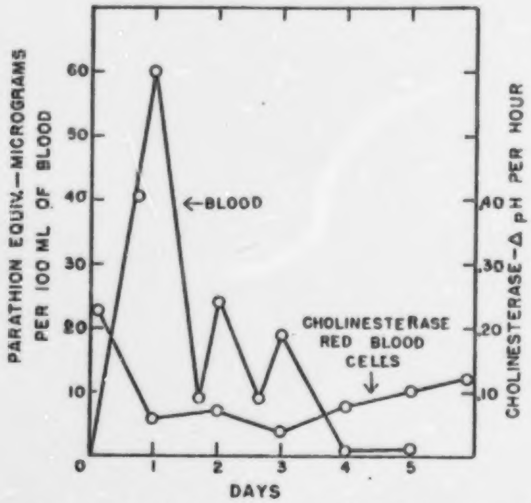

Chart 3.—Parathion equivalent in blood and red cell cholinesterase activity in rabbits. Dermally treated skin area excised 72 hours after dosing.

In another experiment, 125 mg. of parathion was applied dermally to a rabbit, and determinations of cholinesterase activity, as well as radioactivity measurements, were made on blood samples taken at intervals after dosage. Cholinesterase was determined by the method of Michel.[9] Seventy-two hours after dosage, the treated skin area was excised, and the blood assays were continued for two more days. The data obtained are shown in chart 3. It is noted that as the parathion in the blood increases initially, cholinesterase activity decreases. The irreversible nature of the deactivation of cholinesterase is indicated by the slow return toward a normal level after the complete disappearance of radioactivity in the blood stream. In this experiment the urine was again found to be highly radioactive.

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[9] Michel, H. O.; An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity, J. Lab. & Clin. Med. 34:1564, 1949.

The total parathion equivalent excreted in the urine over a period of 15 days after dermal dosing was found to be 5 to 9 mg., or 5 to 7% of the total applied (125 mg.). It should be kept in mind that a high proportion of the parathion applied is never absorbed. The treated skin area was always grossly radioactive at the time of autopsy.

One of the objectives of this study was to determine whether parathion or any sulfur-containing degradation product was stored in organs or tissues. To study this, 1- to 2-gm. samples of organs taken at autopsy were digested in 50-ml. portions of a pepsin-hydrochloric acid mixture. Aliquots of the digest were radioassayed after evaporation to dryness in aluminum pans. Table 2 presents the concentration of active material expressed as parathion found in various fluids and organs. The calculated total quantity of active material in each organ is also shown. None of the organs were perfused, and a part of the parathion equivalent may be due either to the residual blood in the organs or to residual urine in the kidneys.

TABLE 2.—*Concentration and Calculated Total of Parathion Equivalent in Body Fluids and Organs of Two Rabbits on the Fifth and Twentieth Days after Dermal Application*

		Parathion Equivalent			
		Concentration γ per Gm.		Calculated Total Mg.	
	Estimated Weight,	Rabbits *		Rabbits	
Material	Gm.	B	C	B	C
Urine †	125	0.56	4.28	0.0700	0.5400
Blood	240	0.11	3.79	0.0260	0.9100
Bile	1	0.06	0.31	0.0001	0.0010
Liver	85	0.31	0.31	0.0260	0.0260
Fat	168	0.11	0.50	0.0100	0.0870
Muscle	2,700	0.11	0.21	0.3000	0.5700
Brain	7.30	0.13	0.13	0.0010	0.0010
Heart	7.50	0.10	0.21	......	0.0016
Spleen	1.25	0.51	2.02	0.0006	0.0025
Kidney	18.0	Trace	0.22	......	0.0040
			Total	0.4364	2.1581

* Autopsy was done on Rabbit B 20 days after dosing; on Rabbit C 5 days after dosing.
† Urine was obtained from the bladder at autopsy.

It is particularly significant that in Rabbit B, on which autopsy was done on the 20th day, the residual activity in the organs and in the body fat and muscle amounted to less than 9% of the total excreted in the urine over a period of 15 days. Equally significant, even at peak levels in the blood and urine, residual parathion equivalent in the whole body of Rabbit C, on which autopsy was done on the 5th day, was less than 2% of the amount applied. As shown in Table 2, there was five times as much parathion equivalent in the rabbit examined on the 5th day as there was in the rabbit examined on the 20th day after application. These findings, together with the blood and urine assays, indicate that the storage of parathion or a sulfur-containing degradation product is minimal and excretion is prompt.

Attempts were made to identify the chemical nature of the radioactive compounds present in blood and urine after the dermal application of parathion. Samples of blood and urine from a dosed animal were radioassayed, subjected to a high vacuum for several hours, and subsequently reassayed. There was, however, as shown in Table 3, no appreciable loss of activity, indicating that the parathion was bound in some manner or else was degraded to an inorganic state. The pronounced loss of parathion from saline in a relatively short time is typical when parathion remains intact and is not bound. For example, attempts to prepare standards by incorporating the active compound in plastics or similar media failed because of the marked vaporization of parathion from such mixtures. Whether parathion is bound in the blood as such or whether it is in a degraded state is not clear from this work. In the case of urine, however, the activity observed is apparently due to thiophosphoric acid or its ions. Evidence as to the latter is contained in experiments in which urine samples were treated with an anion exchange resin ("amberlite IR-4B," Rohm & Haas Co.). The sample assayed 101,000 cpm before treatment and 18,000 cpm after treatment. On regeneration with 5% sodium hydroxide, a radioactive solution was obtained assaying 48,000 cpm. A portion of this solution was treated with ammonium molybdate, giving a strongly radioactive yellow precipitate, the filtrate being essentially nonradioactive. It seems apparent from these experiments that the thiophosphoric acid moiety appears in large amounts in the urine. Whether degradation of the parathion occurs before or after excretion is not known. Since organic esters of inorganic acids such as parathion are generally very susceptible to hydrolysis, it seems most likely that the parathion is split some time prior to its excretion in the urine.

TABLE 3.—*Radioactivity of Blood, Urine, and Saline Before and After Vacuum Treatment*

Material	Evacuation at < 1 Mm. Hg, Hours	Activity Assay (Cpm)	
		Preevacuation	Postevacuation
Blood smear	8	22.5	21.9
Urine sample	72	5,130	4,944
Parathion in saline	½	3,240	1,767

SUMMARY

A study of the fate of parathion in rabbits treated dermally and intravenously with radioactive S^{35}-labeled parathion has been made. Evidence has been obtained showing that there is very little accumulation of parathion or a sulfur-bearing portion of the molecule in the blood, organs, or tissues. The compound or sulfur-containing degradation products are rapidly excreted in the urine. The excreted moiety appears to be the inorganic residue of parathion and is absorbed by anion exchange resins, from which it can be regenerated and precipitated by ammonium molybdate.

We are indebted to Dr. Paul Ortega for performing autopsies and for preparation of tissue samples. Acknowledgments are also due to Dr. Wayland J. Hayes Jr. for advice and guidance in the course of the study.

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DDT Storage in the US Population

Griffith E. Quinby, MD, MPH, Wayland J. Hayes, Jr., MD, PhD,
John F. Armstrong, and William F. Durham, PhD

Analysis of fat samples from 130 subjects indicated that the general population of the US had a mean storage level of 4.0 parts per million (ppm) DDT and 7.8 ppm 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (DDE) Geographic differences between samples taken in Phoenix, Atlanta, Louisville, and Wenatchee, Wash, were not evident. No difference between levels of DDT or DDE in the general population and in persons living in areas of extensive agricultural use of DDT were noted; however, occupationally-exposed workers had higher levels. Comparison of results of the present study with those of earlier surveys revealed no distinct directional trend in the storage level of DDT or DDE over the years between 1950 and 1962. The DDT storage level for the US was higher than for Canada, England, France, and West Germany, but about the same as for Hungary.

Storage of the chlorinated hydrocarbon pesticides, particularly DDT, in body fat following occupational, environmental, or dietary exposure has been a matter of concern to those interested in evaluating health hazards of the widespread use of pesticides. Surveys of the storage of DDT and its metabolite 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE) in the general population and in specific occupational, environmental, and dietary groups in the US were summarized in our paper[1] reporting the measurement of storage for samples collected during 1954 through 1956. These surveys are also summarized in Table 2. Storage in West Germans,[2] Canadians,[3] Alaskan Eskimos,[4] Hungarians,[5] Britons,[6] and Frenchmen[7] has been reported subsequently.

The present paper reports the results of analyses of samples collected during 1961 through 1962 to determine the current level of DDT and DDE storage in the population of the US.

The development of gas chromatography has made it possible to broaden the objectives of the present study. In addition to the measurement of storage of DDT and DDE, some of the samples in this survey have been analyzed and reported for other chlorinated hydrocarbons: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), dieldrin, mixed isomers of 1,2,3,4,5,6-hexachlorocyclohexane (BHC), aldrin, heptachlor epoxide, and methoxychlor.[8] Only dieldrin and BHC were found in detectable quantities. Samples of the diet representative of the general population have been collected to determine pesticide content of complete meals. Urine samples from approximately one half of the survey patients have been analyzed for bis (*p*-chlorophenyl) acetic acid (DDA), a metabolite of DDT. Results from these segments of the survey will be published separately.

Materials and Methods

Since the results reported in this paper were to be compared with those of our earlier study,[1] materials and methods were kept as nearly the same as possible.

Samples of human adipose tissues were collected in hospitals in Wenatchee, Wash, Louisville, Phoenix, and Atlanta. Although the majority of persons from whom these samples came had lived in these cities over two years, a number had lived until recently in other parts of the US. The great majority (93%) of the samples were collected by biopsy, incidental to routine surgical procedures; a small portion (7%) came from autopsies. A total of 178 persons were studied for DDT and DDE storage. Additional fat tissues were obtained from four persons who had been sampled earlier.

The history of exposure to insecticides was recorded as far back as the patient or best informant could recall. Possible intake of DDT and DDE was noted for consumption of red meat, fowl, eggs, fish, dairy products, and cooking fats. Patients were queried for possible significant occupational exposure. Some applicators had records of their work, and the rest remembered enough to confirm the fact that they had had extensive exposure. Whether each patient lived within 500 ft of areas of agricultural application was determined. Exposure from household or garden applications was recorded as accurately as could be recalled for two or more years. However, in the majority of instances the patient did not know which insecticides had been contained in the proprietary aerosol-dispensing de-

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From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, US Department of Health, Education and Welfare, Wenatchee, Wash, (Drs. Quinby and Durham and Mr. Armstrong) and Atlanta (Dr. Hayes).

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145

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Reprint requests to PO Box 73, Wenatchee, Wash 98801 (Dr. Quinby).

Table 1.—Storage of DDT and DDE in Fat of People in US by Degree of Dietary, Environmental, and Occupational Exposure, 1961-1962

Exposure Group		No. of Cases	Value and Unit		DDT		DDE	
No.	Description				Tissue	Extract	Tissue	Extract
3	General population	130	Range	ppm	0.7-25.4	1.2-48.1	1.7-30.8	2.5-50.7
			Mean ±SE	ppm	4.0±0.31	6.8±0.57	7.8±0.50[*]	13.5±0.82[*]
			Coefficient variable	%	8	8	6	6
4	People with heavy environmental exposure	28	Range	ppm	0.4-16.8	0.9-18.0	0.6-23.7	2.2-41.8
			Mean ±SE	ppm	4.3±0.66	6.5±0.78	7.7±0.92	12.1±1.5
			Coefficient variable	%	15	12	12	12
5	Applicators more than 1 year ago	6	Range	ppm	1.6-9.4	3.5-15.5	9.5-19.0	13.5-28.8
			Mean ±SE	ppm	6.4±1.3	9.3±1.9	14.0±1.6	21.1±2.4
			Coefficient variable	%	20	20	11	11
6	Applicators within last year	14	Range	ppm	2.2-30.3	4.4-42.2	5.1-52.9	8.4-91.6
			Mean ±SE	ppm	10.7±2.1	16.5±2.9	21.6±4.1	33.9±6.3
			Coefficient variable	%	20	18	19	19

[*] Based on 121 samples.

vice, solution, or dust. The user had simply accepted advice that the product was effective in killing a particular pest. However, an occasional informant knew precisely which household or garden insecticides had been applied, but often these persons could not recall how much or what strength had been used.

Each case was classified according to history into exposure groups numbered 1 through 6, arranged in approximate order of increasing exposure. The identity of these exposure groups, which was essentially the same as in the previous survey,[1] was as follows: *group 1*—persons who died before advent of DDT in the US; *group 2*—abstainers from meat; *group 3*—general population (persons eating meat more than 12 times a month and without heavy environmental or occupational exposure); *group 4*—heavily environmentally-exposed residents living within 500 ft (152 meters) of agricultural application, as compared to a maximum of 5,200 ft (1,610 meters) for the earlier survey[1] (For purposes of this study, household or community applications were not considered "heavy environmental" exposure.); *group 5*—former applicators (more than a year ago); *group 6*—applicators (within the last year). For inclusion in this latter group a person had to have applied DDT in some agricultural or other commercial fashion at least twice during the year.

Insufficient numbers of samples in groups 1 and 2 were analyzed in the present study to be of significance.

For the exposure histories of nine of the patients from Louisville, the patients, their next of kin, or other informants could not be contacted. However, after reviewing hospital records of the patients and considering their residence and occupation, these nine individuals were placed in group 3, the general population group. Complete exposure histories had previously been obtained on 30 patients from the same hospital, and none was found to have any type of exposure other than that characteristic of the general population.

No racial selections were exercised. Recorded race included Caucasian, Negro, and American Indian.

Body fat samples as large as practical were collected and preserved in 10% formalin. The usual size of the sample used for analysis was approximately 2 to 4 gm. Fat samples smaller than 2 gm

Table 2.—Storage of DDT and DDE in General Population and in Persons With Heavy Environmental Exposure in US

Group No.	Type of Exposure	Number of Persons and Location	Adipose Tissue			Lipid Extract		
			DDT	DDE	Total[†]	DDT	DDE	Total[*]
			(Averages in ppm)			(Averages in ppm)		
			1954-1956 Survey, Hayes et al[1] and unpublished data					
3	General population	11 (Wenatchee, Wash)	6.2±1.5	7.9±1.6	15.0±3.1	7.6±1.6	10.1±1.8	18.8±3.6
		50 (Savannah, Ga)	4.7±0.28	5.7±0.37	11.0±0.63	6.6±0.37	8.2±0.49	15.7±0.83
		61 (US)	4.9±0.35	6.1±0.42	11.7±0.77	6.8±0.42	8.6±0.52	16.3±0.93
			1961-1962 Survey[†]					
		25 (Atlanta)	4.2±0.55	6.7±0.64	11.7±1.0	7.8±1.2	12.3±1.0	21.3±2.1
		16 (Phoenix)	5.3±1.3	13.2±2.3	20.0±3.7	7.3±1.6	18.2±2.9	27.5±4.7
		27 (Louisville)	4.4±0.98[‡]	6.5±1.1[‡]	11.6±2.0[‡]	8.8±2.6[‡]	13.8±2.9[‡]	24.3±5.4[‡]
		62 (Wenatchee, Wash)	3.4±0.29	7.5±0.60	11.7±0.92	5.7±0.47	12.7±1.0	19.9±1.5
		130 (US)	4.0±0.31	7.8±0.50	12.7±0.81	6.8±0.57[§]	13.5±0.82[§]	21.9±1.36[§]
4	Heavy environmental exposure		1954-1956 Survey, Hayes et al[1]					
		110 (US)	6.0±0.26	8.6±0.48	15.7±0.74	7.9±0.41	11.0±0.62	20.2±0.99
			1961-1962 Survey[†]					
		28 (US)	4.3±0.66	7.7±0.92	12.8±1.5	6.5±0.78	12.1±1.5	19.9±2.2

[*] Expressed as DDT equivalent.

[†] This paper.

[‡] Based on 18 samples by Mattson modification of Schechter-Haller method. Remaining 9 samples based on Schechter-Haller reading of extract prepared for gas chromatography without lipid determination.

[§] Based on 121 samples.

Table 3.—Trend of DDT and DDE Deposited in General Population of US

Collection Period	Type of Exposure	Method of Analysis	Number of Persons	PPM in Adipose Tissue: DDT Range	DDT Mean	DDE Range	DDE Mean
<1942 [1, 10]	Before DDT	Modified S-H*	13	No greater than reagent blanks			
1950 [11]	Probably general population, but detailed history not available	Original S-H*	75	0-20+	5.3		
1951-1952 [10]	General population mostly, but also some formulators, applicators, spraymen, and residents in treated areas	Original S-H*	74	0-15	5.4	...	...
1953-1954 [12]	General population (federal prisoners)	Modified S-H*	49	3-13	7.4	2-26	11.2
1954-1956 [1]	General population	Modified S-H*	61	2-12	4.9	2-13	6.1
1962-1963 [13]	Probably general population, but exposure history not obtained	GLC†	282	0.1-19.0	2.9	0.5-39.0	7.4
1961-1962‡	General population	Modified S-H*	130	0.7-25.4	4.0	1.7-30.8	7.8

*Schechter-Haller colorimetric method.[9]
†Gas-liquid chromatography.
‡This paper.

were not analyzed unless there was special interest in the patient, such as an unusual type of exposure history.

All analyses for DDT and DDE reported in this paper were carried out by the Schechter-Haller spectrophotometric method,[9] as modified by Mattson et al.[10] Although the limited accuracy of this chemical method for detecting trace amounts of DDT and DDE in quantities less than 5μg was realized, all samples which produced recognizable Schechter-Haller colors were accepted at their calculated values. There were no samples which failed to give a visible blue, red, or intermediate color in the Schechter-Haller reaction.

All of the values for the total DDT-derived material (DDT plus DDE) in this paper have been expressed in terms of a DDT equivalent.

After most of the analyses in this study had been completed, the fact was established that the Schechter-Haller method does not quantitatively distinguish the various isomers and ratios of isomers of DDT and DDE, as is possible by gas chromatography.[8] The Schechter-Haller method (with or without modification) is now known to reflect up to 88% higher DDE levels and down to 29% lower DDT levels than are demonstrable by more precise analysis in certain ratios of isomers in fat.[8] Nevertheless, the Schechter-Haller method has been employed for so long that it is important for purposes of comparison with earlier studies for additional data to be reported in this form, at least until there is more information based on the newer techniques.

Results

The levels of DDT and DDE in people with differing degrees of dietary, environmental (residential), and occupational exposure as determined in the present survey are presented in Table 1. As in the earlier survey, the storage levels of DDT and DDE for each group were proportional to the exposure level. The average storage in adipose tissues of the general population (group 3) was 4.0±0.31 parts per million (ppm) of DDT and 7.8±0.50 ppm of DDE, with a total of 12.7±0.81 ppm of both chlorinated hydrocarbons expressed as DDT. The storage levels of DDT and DDE in people living in an area with heavy environmental exposure (group 4) were essentially the same as those for the general population. Agricultural applicators (group 6) and former agricultural applicators (group 5) showed levels significantly higher than those of people without occupational exposure.

In Table 2 the mean storage levels of these compounds are shown separately for the general population groups of the various cities sampled and for the environmentally-exposed group in both the earlier (1954-1956) survey [1] and the present study. There was little variation in DDT level for the general population group between the four cities. Although the Wenatchee, Wash, area has at least as heavy, if not the heaviest, use level for DDT of any of the geographical areas surveyed, the DDT storage for this subgroup was the lowest for the cities tested. The DDE in adipose tissue was almost twice as high in Phoenix as in the other cities. However, when calculated on an extractible lipid basis, much of this difference disappeared, indicating that the difference was primarily due to variation in lipid content of the adipose tissue analyzed.

As also shown in Table 2, there appears to be no difference now between levels of DDT and DDE storage in the general population (group 3) and the levels in persons living in or near areas of extensive agricultural use of DDT (group 4). This finding is in contrast to the result in the earlier study in which the DDT storage level for the environmentally-exposed group was significantly higher than that for the general population. This different result occurred in spite of a more stringent requirement in the present study for inclusion in the environmentally-exposed group. The difference may be due to a decreased use of DDT in the test area from which the environmentally-exposed group was drawn. Records and estimates by the most qualified observers indicate that the use of DDT has decreased more than half in frequency and ex-

Table 4.—Comparison of Levels of DDT and DDE in Fat of People in General Population Tested in Surveys Conducted from 1951-1962, Wenatchee, Wash

Case No.	Compound	Ppm in Adipose Tissue 1951-1952	1954-1956	1961-1962
1	DDT	3.0	....	4.0
	DDE	....	....	3.8
2	DDT	<10	....	3.1
	DDE	....	....	6.5
3	DDT	....	4	2.1
	DDE	....	4	4.8
4	DDT	....	6	2.2
	DDE	....	12	6.3

tent in the apple-producing areas of the Wenatchee Valley from which most of these samples came. During the 1961-1962 survey all but seven of the 28 people in group 4 were from the Wenatchee Valley.

As shown in Table 2, there is no increase in DDT storage level for the general population group (4.0±0.31 ppm) in 1961 through 1962 as compared with 1954 through 1956 (4.9±0.35 ppm). The apparent decrease has no statistical significance. The averages for DDE in the latest (1961-1962) survey suggested a possible rise, especially when calculated on a lipid basis. However, careful examination of the data indicated that much of this difference occurred in a few of the 16 specimens from Phoenix with the highest DDE levels. Careful rechecking of histories of the patients from Phoenix uncovered no previously undisclosed exposures. However, there did appear to be a considerably longer and heavier household use of sprays for fly control in the Phoenix area than in the other areas studied. For the environmentally-exposed group, there was no increase in DDT or DDE storage level in 1961 through 1962 as compared with 1954 through 1956.

Table 3 presents the results of all the DDT surveys which are known to have been carried out in this country during the last 14 years. These results reflect storage levels before DDT was used and since DDT was introduced for military use in 1943 and commercial use in 1946. The earliest survey (1950) indicated a mean DDT storage level for the general population of 5.3 ppm.[11] Methods available at this time did not include determination of DDE content. With the exception of the federal prisoners who were noted by Hayes et al[12] in 1953 through 1954 to have a mean DDT storage level of 7.4 ppm, all general population groups in this country have in subsequent studies shown storage levels about the same as or lower than that found in the 1950 survey. Hoffman et al[13] have recently reported a relatively low DDT storage level (2.9 ppm) for a group of 282 persons, presumably from the general population. However, these values which were obtained by the gas chromatographic method of analysis must be interpreted in the light of the differences in DDT and DDE storage levels between colorimetric and gas chromatographic methods noted by Quinby and Dale.[8] Thus, it can be seen that there has been no change since 1950 in levels of either DDT or DDE that cannot be attributed substantially—if not entirely—to improved methods of analysis.

The trend of DDT storage levels in the general population, as illustrated by the frequency distribution of subjects at various ranges of storage, has also been evaluated. In the present survey, there were no samples which did not contain detectable levels of DDT. There was only one sample which contained over 20 ppm of DDT. Of the samples 75% contained between 1 and 5 ppm. In comparing these values with frequency distributions from earlier years it appears that there has been a decrease over the years in the number of persons with very low or very high storage levels of DDT. More recent studies show a greater concentration of figures near the mean value. This difference in distribution may be explained by a more regulated use of DDT in agriculture and/or differences in chemical method.

The stability of DDT storage level has occurred in the face of continuing large-scale use of DDT. Apparently, "disappearance" figures are the best available measure of the use of DDT in this country. For the period 1950 through 1962, these figures varied from a low of 45,117,000 lb in 1954 to 78,682,000 lb in 1959 without any definite trend to increase or decrease.

It is interesting to consider the number of subjects in a given exposure group which one would need to study in order to determine if differences in DDT storage existed. In order to estimate the sample size necessary for a specified precision, one may use the formula given by Sukhatme:[14]

$$N = \frac{Z(\delta)^2\ S^2}{E^2\ \bar{X}^2}$$

Where

$\bar{X}$ = the mean from a preliminary sample,

S^2 = the variance from a preliminary sample,

$Z(\delta)$ = the standardized normal deviate when the level of confidence desired is 1-δ, and

E = the desired precision.

In the present set of 130 samples from the general population, the mean for DDT was 4.0 and the variance was 12.7. The precision obtained at the 95% level was 15%; that is, one would expect to find means varying between 3.4 and 4.6 in 95% of tests using 130 samples each. Further calculation

Table 5.—Concentration of DDT-Derived Material in Adipose Tissues of General Populations of Different Countries

Country	Years of Survey	Number of Samples	DDT, Ppm	DDE as DDT, Ppm	Total as DDT, Ppm	DDE as DDT, %*
Canada	1959-1960[illegible]	62	1.6	3.7	5.3	69.8
England	1961-1962[illegible]	131			2.2	
France	1961[illegible]	10	1.7	3.5	5.2	67.3
Hungary	1960[illegible]	50	5.7	6.7	12.4	51.3
US	1961-1962†	130	4.0	8.6	12.6	68.3
West Germany	1958-1959[illegible]	60	1.0	1.3	2.3	56.5

*Calculated as follows: DDE (as DDT in ppm) × 100/DDE (as DDT in ppm) + DDT (ppm).

†This paper.

shows that a precision of 20% could be obtained in 95% of tests by use of 76 samples. Finally, it may be calculated that the use of only 10 samples would permit a precision of approximately 55% in 95% of tests. Even with a precision of only 55%, values for the general population could easily be distinguished from those for people in this country who abstain from meat or those for workers with moderate occupational exposure, not to mention values from workers with heavy occupational exposure where a storage level as high as 648 ppm has been observed.[12]

One of the best measures of the trend of DDT deposits in the fat of the general population should be multiple biopsy of the same individual over the years. It is not an easy matter to acquire such samples in quantity. However, during the course of the last three surveys from 1951 through 1962, it has been possible to obtain multiple samples from four individuals as shown in Table 4. Results of the study of these multiple biopsies show slight changes in both directions, but no indication of increased storage is noted. In comparing the results obtained in 1951 with those of later years, one should consider the higher limit of detectability in 1951. In effect, samples reported as below detectable limits in 1951 would be reported as positive in later years. Thus, these limited data on four individuals are consistent with the results of the surveys of larger groups of the general population, which show no significant change in the levels of DDT since it was first measured in 1950.

One of the samples from the general population came from a 6-month-old bottle-fed boy whose extractible lipid levels for DDT (6.2 ppm) and DDE (16.5 ppm) were well within the range for the general population. Thus, by the 6th month of life it is possible for a child to deposit at least the average concentration for the general population. In view of these findings, it is interesting to compare the results of the DDT human volunteer study reported by Hayes et al.[12] The subjects in this study had essentially reached a plateau level of DDT storage after six months of dosage. Later unpublished work by Hayes and his co-workers indicates that loss of DDT from storage is very slow after dosage is discontinued.

The recent report by the Surgeon General's Advisory Committee on Smoking and Health has called attention to the health hazard involved in cigarette smoking. A number of persistent pesticides, including DDT, are used in tobacco culture. The possible contribution of these residues to the hazard from smoking cigarettes should be explored. Evaluation of the relationship between DDT storage and smoking has been limited in the present study to the male segment of the general population group. Reliable data on smoking habits was obtained for 13 of these men. Only those subjects were classified as smokers who gave a history of extended, heavy smoking of cigarettes. Mean DDT and DDE levels were determined for the smokers (four subjects) and nonsmokers (nine subjects). Mean tissue storage levels for DDT were 4.0±0.44 ppm for smokers and 3.1±0.80 ppm for nonsmokers. Mean tissue storage levels for DDE were 7.4±0.98 ppm for smokers and 3.1±9.80 ppm for nonsmokers. As determined by Students' *T*-test, there was no significant difference ($P>0.4$) between smokers and nonsmokers with regard to storage of DDT or DDE.

As cited in the introduction and shown in Table 5, the levels of DDT in the population of five countries other than the US have been determined. Storage of DDT in England, West Germany, France, and Canada is lower than in the US. Only in Hungary did the level of DDT storage equal or exceed that of the US.

There is close agreement between the reports from various countries regarding the proportion which DDE contributes to the total storage expressed as DDT. The five reports indicate that approximately 65% of the DDT-derived materials is DDE. The fact must be kept in mind that in all probability the entire amount of this DDE was not derived in vivo in man, but some of it was ingested preformed in foods containing DDE.

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35

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Dinitroorthocresol Exposure from Apple-Thinning Sprays

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The sodium salt of dinitroorthocresol (DNOC) has been used agriculturally for a variety of purposes. As a fungicide it has been used as a so-called "eradicant" spray to kill spores during the dormant season. It has proved quite effective as a weed killer. It has been used as an insecticide against a variety of pests, including mites, where it serves as an ovicide as well as an adulticide, and locusts. It is finding increasing use in apple orchards as a means of chemically thinning blossoms.

Though the Food and Drug Administration[1] has set a zero tolerance for DNOC as a residue on raw agricultural produce and the U. S. Department of Agriculture[2] has insisted on a poison label on agricultural formulations of it, nevertheless, farmers, fieldmen, and insecticide salesmen in this country have commonly considered it to be one of the safer materials as compared, for instance, with the organic phosphorus insecticides. This reassuring attitude is in sharp contrast to views held in Europe, where there have been a number of deaths among spray operators who were applying this material. The pattern of one or two deaths each year over a period of several years is remarkably similar to the record for organic phosphorus compounds in this country. Six of the eight cases reported by Bidstrup and Payne[3] are particularly interesting, since they involved no accident, gross carelessness, or other massive acute exposure immediately preceding death. Rather, each illness followed at least two weeks of chronic exposure with few, if any, notable incidents. This is in direct contrast with deaths from parathion or tetraethyl pyrophosphate, which have characteristically followed a single massive dose usually resulting from accident or gross carelessness and frequently without significant previous exposure.

The intensive use of DNOC or its sodium salt in local areas in this country over short periods of time has resulted in persistent yellow stains on the hair, hands, and feet of many workers but has apparently produced no more serious consequences. It was therefore felt desirable to study exposure to the sodium salt under conditions in which it is used as a chemical blossom thinner in the Wenatchee Valley of Washington. The object of this study was to determine whether or not a serious health hazard existed under conditions of use in this area.

The material most commonly used for blossom thinning in the Wenatchee Valley is a slurry containing 19% of the sodium salt of dinitroorthocresol, 5% of sodium butyl naphthalenesulfonate, and 2% of sodium chromate, together with small amounts of sodium chloride and sodium sulfate. The standard recommendation is to apply 1 to 2 gal. of this concentrate per acre in 500 to 1200 gal. of water, depending upon the spray equipment used. The timing of the application

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is rather critical, and it is unusual for any one operator to use this material for more than five days or for more than six hours on any one day. The timing of the spray must coincide with peak bloom or fall within a very few days thereof, which means the dates in the Wenatchee Valley are usually within two weeks of May 1. The temperatures of the Wenatchee Valley vary greatly in different locations, and there is a very wide fluctuation at any one station, with a recorded range for April and May of 21 to 96 F. The average daily maximum for April is 59 F, and that for May is 66 F. The corresponding average minima are 37 and 44 F. The humidity is generally very low, though occasional rains are encountered. Since rain within 48 hours of spraying with Na-DNOC does considerable damage to foliage, some effort is made to avoid spraying when rain seems imminent. Rather strong, drying winds are common, but spraying is halted at any time that the wind rises appreciably.

Table 1.—*Dermal Exposure to Na-DNOC per Hour of Spraying*
Calculated on Basis of Short Sleeves, No Gloves, and Open Neck

Unclothed Body Part	Location of Pads	Pads Analyzed, No.	Body Area, Sq. Ft.	Pad Recovery, Mg./Sq. Ft./Hr.	Total Calculated Exposure, Mg./Body Part/Hr.
Face	Shoulders	76	0.70	20.3	14.2
Hands	Forearms	76	0.87	20.9	18.2
Forearms	Forearms		1.30	20.9	27.2
Back of neck	Back of neck	74	0.12	16.3	2.0
Front of neck "V" of chest	Front of neck	74	0.16	9.9	1.6
Total		300	3.15	....	63.2

METHODS AND RESULTS

An estimate of dermal exposure was obtained by exposing absorbent pads on forearms, shoulders, thighs, and back and front of the neck of the operators during one or more complete spraying cycles. The procedure has been reported in detail in connection with its use in estimating parathion exposure.[4] The pads were then extracted with water in a Soxhlet apparatus until no color was visible in the pad. The intensity of the color of the extractant was read in a Model B Beckman spectrophotometer at 370 mμ and compared with a standard curve. The results of these analyses, as presented in Table 1, indicate an average dermal contamination of 63.2 mg. per hour of exposure, assuming that the operator wore a shirt with short sleeves and an open collar and that he wore no hat or gloves and assuming further that his clothing gave complete protection of the area covered. Respiratory exposure was estimated by analyzing the filters from MSA-type respirators worn during one or more complete cycles. The average for 74 respirator samples was 0.4 mg. per hour of exposure. Thus, the respiratory exposure appears to be less than 1% of the dermal and should be negligible.

To provide an estimate of the quantity of Na-DNOC actually absorbed by the spray operators, urine samples were collected at various intervals before, during, and after the exposure period and analyzed using the procedure described by Parker.[5] Of 183 urine samples analyzed, only 5 contained as much as 0.5 ppm of the sodium salt (the lower limit of sensitivity of the method). These five samples contained from 0.6 to 1.3 ppm of Na-DNOC, with an average of 0.8. Harvey and associates [6] found detectable amounts of DNOC in the urine of all five volunteers in the first specimen passed after ingestion of a 75 mg. dose of the toxicant. They reported little correlation between DNOC concentration in urine and that in blood. The excretion of DNOC in urine was found by the abovementioned authors to vary between 1 and 2 ppm following a 75 mg. dose.

In an effort to find a more sensitive estimate of DNOC absorption, blood samples were drawn from several of the operators whose exposure was of exceptionally long duration, within 24 hours of their last exposure and again one or two weeks later, but in any event before any subsequent exposure to parathion or other pesticide that might interfere with the analysis. The plasma fraction of these bloods was extracted and analyzed by the same technique used for urine. Blood samples taken from persons who had no known exposure to pesticides were similarly treated and used for blank determinations. The results of these analyses are presented in Table 2. The highest concentration observed was 4.3 ppm.

Table 2.—*Concentration of Na-DNOC Found in Plasma of Spray Personnel at Intervals After Last Exposure*

Case No.	Approx. Total Exposure, Hr.	Conc. of Na-DNOC in Plasma, Ppm, Days After Last Exposure		
		1	7	11
1	48	...	2.2	...
2	35	2.5	4.3	1.2
3	35	4.0	2.3	<1.0
4	35	1.4	3.3	2.7
5	10	...	1.6	...
6	5	...	2.4	...

COMMENT

Harvey and his associates[6] showed that daily oral doses of 75 mg. of DNOC produced blood levels in human volunteers from 15 to 20 ppm after three to five days. After this range of concentration had been reached, additional doses caused temporary increases of concentration which frequently were associated with symptoms and which, on one occasion, reached a level of 48 ppm. The same authors presented a few cases involving experimental dermal dosage with Na-DNOC, which clearly demonstrated absorption and which appeared consistent with the findings from animal experiments that DNOC is absorbed less readily through the skin than through the alimentary or respiratory tracts.

The average measured dermal exposure reported in this paper is 63.2 mg/hr., and the corresponding value for respiratory exposure is 0.4 mg/hr. It appears premature to speculate on the relation between these dosages and the oral dosages used in the study of volunteers except to state that our observations are consistent with the conclusion that DNOC is absorbed less readily through the skin than through the gastrointestinal tract. Certainly the highest DNOC blood level (4.3 ppm) observed following occupational exposure was lower than 20 ppm which the work of Harvey and his associates suggests is near the critical level. No symptoms were reported in any of the subjects in the Wenatchee Valley.

Bidstrup and Payne state that it is the custom for men spraying cereal crops with DNOC for weed control in Great Britain to work long hours. The stock solution reported to be used in Great Britain contained up to 50% DNOC as compared with a standard 19% Na-DNOC used in the Wenatchee Valley. The dilute spray reported to be commonest in Great Britain contained 0.5% to 0.8% DNOC, though one of the fatal cases used 2% and two of the others used 0.8% to 1.25% material in their spray tanks. In the Wenatchee Valley the recommended dilute spray is 0.02% to 0.04%. Three of the fatal cases reported from Great Britain had applied from 900 to over 1500 lb. of actual DNOC during the season in which their deaths occurred, whereas the largest amount of actual DNOC known to be applied by any one spray operator in the Wenatchee Valley during one season was 50 to 100 lb.

Thus, in contrast to England, where DNOC has proved a hazard, the use of DNOC in the Wenatchee Valley appears to involve shorter working hours, more dilute stock solutions, more dilute spray solutions, and the application of much less of the compound during a season by any one worker. The measured exposure to DNOC of spray operators in the Wenatchee Valley and their urinary and blood levels of the compound indicated that these operators are not subject to a significant hazard in connection with the recommended use of DNOC for thinning blossoms.

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152

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DDA Excretion Levels

Studies in Persons With Different Degrees of Exposure to DDT

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THE POTENTIAL health hazard of pesticides in general and of the persistent chlorinated hydrocarbon insecticides in particular has been the subject recently of much public interest.[10] An important aspect of study of the health hazard of these materials is an evaluation of the magnitude of exposure sustained by the worker in his occupational contact during manufacture, formulation, or application; by the resident who receives drift of spray or dust into his home when the pesticide is used nearby; and by the individual in the general population who consumes residues of these pesticides in his daily diet.

The magnitude of exposure to DDT has been determined by measurement of DDT and DDE storage levels in body fat[8] or, less commonly, by estimation of urinary excretion levels of the relatively water-soluble metabolite DDA (dichlorodiphenyl acetic acid).[2] It is, of course, much easier to obtain urine samples for DDA analysis than to procure surgically the fatty tissue required to measure DDT and DDE storage levels.

Storage of DDT and DDE has been determined in the general population, in meat abstainers, in various environmental and occupational groups,[7] and in volunteers given known oral doses of DDT.[5,6] DDA excretion has been measured previously in workers in manufacturing and formulating plants[9] and in volunteers given known oral doses of DDT.[5,6]

The present study involved measurement of the DDA excretion levels in persons in the general population who ate an average diet and had no environmental or occupational exposure to DDT. Excretory levels of a small number of meat abstainers and of persons who had been environmentally or occupationally exposed to DDT are also included.

The work described in this paper is a component part of a more comprehensive study. The complete project involves determination of DDT and DDE storage levels in body fat in the general population and in specific environmental and occupational groups, measurement of DDT and DDE content of complete prepared meals, and determination of DDT and DDE excretion in milk by nursing mothers. These surveys have been correlated by obtaining as many of the samples as possible from the same individuals. For example, a number of the subjects in the present study also contributed fat for determination of DDT and DDE storage levels. The results of the other segments of the complete DDT study will be published separately.

Materials and Methods

A total of 107 urine samples were analyzed for DDA content using the procedure of Cueto et al.[3]

Each subject who contributed a urine sample was classified according to history into one of several exposure groups numbered in approximate order of increasing exposure. The identity of these exposure groups was essentially the same as that used in the previous survey of DDT and DDE storage levels.[7] The identity of these groups from whom samples were obtained for this study is as follows:

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TABLE 1.—*Excretion of DDA in Urine of People in the United States with Regard to Dietary, Environmental, or Occupational Exposure to DDT*

Exposure Group No.	Exposure Group Description	No. of Cases	Range of DDA Concentration in Urine (ppm)
2	Abstainers from meat	4	<0.02
3	General population	79	<0.02-0.35
4	People with heavy environmental exposure	13	<0.02-0.11
5&6	Agricultural applicators	11	<0.02-0.17

Group 2: Abstainers from meat (4 cases).

Group 3: General population (persons eating meat more than 12 times a month and without heavy environmental or occupational exposure) (79 cases).

Group 4: Heavily environmentally exposed residents living within 500 feet (152) meters of agricultural application (13 cases).

Group 5: Former applicators (more than a year ago) (8 cases).

Group 6: Applicators (within the last year). For inclusion in this classification a person had to have applied DDT in some agricultural or other commercial fashion at least twice during the past year (3 cases).

No samples in Group 1 (persons who died before the advent of DDT in the United States) were available for analysis in the present study. The numbers of subjects in Groups 5 and 6 were small and the results for them are considered together.

Twenty-three of the individuals in the general population group in this paper also contributed fat for the determination of DDT and DDE storage levels in the general population in a related study.[11]

Results and Comment

The excretion of DDA in urine by people in various dietary, environmental, and occupational exposure groups with regard to DDT contact is shown in Table 1.

The DDA excretion levels for the general population group ranged from below the experimental limit of the analytical method, which was about 0.02 ppm, to 0.35 ppm. Of the 79 samples in this group which were tested, 56 samples (74%) did not contain detectable quantities of DDA. The results shown in the table did not indicate any important difference in DDA excretion level between the general population and any other group studied. It is entirely possible that the DDA excretion level in meat abstainers is lower than that for the general population; however, it is impossible to reach a firm conclusion on the basis of the small number of samples obtained from meat abstainers.

Twenty-three persons from the general population group also contributed fat in the study to

TABLE 2.—*Excretion of DDA and Storage of DDT by People of the United States as Determined by Degree of Dietary, Environmental, Occupational, or Investigational Exposure*

Type of Exposure and Name of Group	DDA Excretion			DDT Storage			Reference
	No. of subjects	Range, ppm	Mean ppm±SE	No. of subjects	Range, ppm	Mean, ppm±SE	
Average exposure							
Volunteers (controls)	8	<0.05	—	8	2.9-9.3	6.9±0.76	6
Same	8	<0.02-0.07	—	10	2.6-5.8	3.7±0.37	5
General population	23	<0.02-0.18	—	23	0.7-8.9	3.0±0.4	This paper
Heavy environmental exposure							
Residents near treated fields	—	—	—	110	2-14	6.0±0.3	7
Same	13	<0.02-0.11	—	—	—	—	This paper
Same	—	—	—	28	0.4-16.8	4.3±0.7	11
Light occupational or investigational exposure							
Agricultural applicators	11	<0.02-0.17	—	—	—	—	This paper
Same	—	—	—	30	3-37	14.0±1.5	7
Volunteers (3.5 mg/man/day)	2	0.10-0.42 *	0.21±0.06 *	2	26-33 †	29.5±3.5 †	6
Same	6	0.06-1.98 ‡	0.23±0.04 ‡	6	39-76 §	50.2±5.6 §	5
Heavy occupational or investigational exposure							
Formulating plant workers	40	0.12-7.56	1.71±0.23	—	—	—	9
Same	—	—	—	2	122-648	—	6,8
Volunteers (35 mg/man/day)	6	0.60-9.67 *	2.46±0.53 *	6	68-367 †	231±42.9 †	6
Same	6	0.18-9.21 ‡	3.09±0.02‡	6	105-619 §	281±79.5 §	5

* Based on all samples after 35th week of dosage.

† Based on the latest samples from each man (11 months or more after dosage started in each instance).

‡ Based on all samples from the 35th through the 93rd week after dosage started.

§ Based on samples taken 21.5 months after dosage started.

determine the storage level of DDT and DDE. The DDA excretion levels for this subgroup ranged from <0.02 to 0.18 ppm. Sixteen samples (70%) from this subgroup did not contain detectable quantities of DDA. Thus, the distribution of DDA excretion levels for the subgroup contributing fat was about the same as that for the entire group studied. The 23 individuals in this subgroup had mean storage levels of 3.0 ppm for DDT and 7.3 ppm for DDE. These values may be compared with levels of 4.0 ppm for DDT and 7.8 ppm for DDE which were determined for the entire general population group of 130 persons.[11] There was no important difference between the DDT or DDE levels for the subgroup and for the entire group, thus indicating that the smaller population sampled for DDA excretion was representative of the entire group which was studied for DDT and DDE storage in fat.

The results of the present study of DDA excretion for the general population and for various environmental and occupational groups are shown in Table 2 in relation to other published studies in which this parameter has been measured. DDT storage levels for these groups are also shown when available.

The absence of any detectable difference in DDA excretion levels among groups with light to moderate exposure has been noted above. However, the DDT storage values are distinctly different for two of these groups—the general population and agricultural applicators—as can be seen in the Table. The reason for the difference between these groups in the case of storage and for their similarity in the case of excretion is not clear. It may well be that further and more exhaustive study of the pharmacodynamics of the equilibrium between DDT stored in fat and DDA excreted in urine will provide the explanation. There seems to be some indication that the DDA excretion level can approach the general population value, while the DDT storage level in fat is still significantly elevated.

Thus, the present method of measurement of DDA excretion does not seem to be precise enough to indicate differences in this parameter correlated with observed intergroup differences in DDT storage level within the relatively narrow dosage range associated with light to moderate exposure. There is a general correlation between DDA excretion and DDT storage for groups with widely different degrees of exposure. However, the rate of change of these two parameters with increase in DDT exposure does not seem to be the same. DDA excretion apparently increases disproportionately at the higher exposure levels.

In view of the above-noted lack of any detectable difference in DDA excretion level among groups with light to moderate exposure, no importance can be attached to the fact that there was reasonably good correspondence among the three studies (Table 2) of DDA excretion in the general population. The same conclusion applies to the failure to detect an effect of environmental exposure to DDT (Group 4) on the excretion of DDA. It will be recalled that even the difference in storage between Group 4 and the general population (Group 3) is small[7]; in fact, the most recent study failed to demonstrate any difference.[11]

Based on an average daily urine output of 1.5 liter and a DDA excretion level range of from <0.02 to 0.35 ppm, calculations indicate that the daily DDA excretion for the general population was between <0.03 and 0.53 mg. Thus, about 75% of the general population excrete less than 0.030 mg of DDA per day or the equivalent of less than 0.038 mg of DDT. It has been found by the analysis of typical meals that the average person in this country consumes about 0.038 mg of DDT per day in his food.[8] Thus, the results of these two studies are not inconsistent. However, it is apparent that present data on the urinary excretion levels of DDA in the general population are insufficient to support any statement concerning the fraction of the daily DDT intake which is accounted for as urinary DDA.

There is a need for a more sensitive method for determination of DDA excretion in urine. This need is particularly great with respect to the general population who excrete quite low levels of this metabolite in association with their low-level exposure to DDT. It is hoped that gas chromatographic methods will provide the solution to the problem of greater sensitivity. However, the acidic nature of the DDA molecule seems to interfere with its passage through the chromatographic column. It has been suggested that esterification of the DDA molecule could be carried out to cut down reactivity and thus aid passage through the column.

DDA is much less toxic than DDT and would appear to contribute little to the acute toxic hazard of the parent compound. The acute oral LD_{50} values to male white rats are 740 mg/kg for DDA and 113 mg/kg for DDT.[4] However, the long-term effects of DDA have not been well studied.

Bleiberg and his colleagues have recently shown that in the rabbit DDA inhibits choline acetylation, apparently by blocking acetate activation, and decreases intestinal motility.[1] These studies were carried out in in vitro systems or on isolated organs. Similar effects have not yet been reported in whole animals.

Summary

The excretion of DDA in urine by people in various dietary, environmental, and occupational exposure groups with regard to DDT contact was determined.

The DDA excretion levels for the general population ranged from below the experimental limit of the analytical method (0.02 ppm) to 0.35 ppm. Of the 79 samples in this group which were tested, 56 (74%) did not contain detectable quantities of DDA.

There was no indication that any important difference in DDA excretion level existed among the general population, meat abstainers, or people with heavy environmental or light occupational exposure. However, striking differences in DDA excretion exist for groups with widely different degrees of exposure.

A more sensitive method for determination of DDA excretion levels in urine is needed, since the present procedure gives values below the limit of sensitivity for about three fourths of subjects from the general population group.

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TOXICOLOGY PROGRAM
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EATING SYSTOX-TREATED FRUIT UNDER CONTROLLED CONDITIONS[1]

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One of the more interesting developments in agricultural insecticides in recent years was the discovery that certain organic phosphorus insecticides are absorbed by the plant and translocated through the vascular system of the plant, retaining their toxicity to certain insects in locations often at some distance from the point of application. The potential value of such "systemic" insecticides does not require elucidation; however, the potential toxicity of their residues justifies more consideration.

The residue problems that have been of greatest concern in the past are largely those of surface deposits. Such deposits are reasonably susceptible to weathering and possess at least the potential of being wiped or washed off, if not in harvesting, perhaps in packing or processing. Deposits that may occur in the flesh of the fruit can be removed only by the metabolism of the fruit, by the natural disintegration of the insecticide itself under rather protected conditions, or by some type of processing.

One systemic insecticide that is of considerable interest in the fruit-growing areas of Washingon is marketed under the trade name of "Systox."[2] Chemically it is the diethoxythiophosphoric ester of 2-ethyl mercaptoethanol. The accepted common name is "demeton."

Unfortunately, rather little is understood about the metabolism of demeton. It is now generally accepted that many of the organic phosphorus insecticides undergo chemical changes associated with the increased toxicity fairly rapidly when absorbed by either plants or animals. Therefore, the nature of these metabolites is of great importance to an understanding both of insecticidal effectiveness and of toxicity to man. For instance, even if the exact amount of unchanged demeton remaining on and within the fruit at harvest time could be determined chemically, this figure would not be an adequate index to the potential hazard of the residue.

Demeton as marketed and applied is known to be nearly as toxic to warm-blooded animals as is parathion or tetraethyl pyrophosphate. Likewise, it is reasonable to assume that it can be safely handled and applied by following the same safety precautions as those recommended for parathion. Therefore, it was registered for use on certain nonfood crops or nonbearing trees several years ago. But the lack of knowledge regarding its metabolism delayed registering its use on food crops. Even now the chemistry of the deposits is not fully understood. No specific analysis for demeton in the tissues of plants is available. The evidence indicates that technical demeton, in common with other organic phosphorus insecticides, owes its toxic properties to its ability to inhibit cholinesterase activity. A nonspecific method of residue determination based on in vitro cholinesterase inhibition has been applied to demeton-treated fruit. This procedure is carried out by mixing a fruit extract with a known quantity of standard cholinesterase. After a suitable incubation period, a cholinesterase activity determination of the mixture will show the effectiveness of the extract in inhibiting cholinesterase. Comparison of standards permits expressing the results in terms of the amount of technical demeton which would have been required to produce this amount of inhibition under the same conditions. For convenience, the results of such determinations are expressed as so many parts per million of demeton-equivalent.

Demeton-treated fruit has been fed to experimental animals, the blood of which was then analyzed for cholinesterase activity. With fruit treated according to reasonable recommendations no significant depletions were found in the blood enzyme levels of experimental animals. As a result of numerous such tests, the U. S. Department of Agriculture registered in May of 1954 labels for demeton advising its use on bearing apple trees, providing that the concentration is not more than one pint of the twenty-one percent material per hundred gallons and that the application is made not later than 21 days before harvest.

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[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.

[2] Chemagro Corp., New York, N.Y.

However, it is well known that different species of experimental animals may not have the same degree of susceptibility to any given toxicant. Even such closely related species as the Norway rat and the roof rat may and do react differently. Therefore, the only way to be sure how susceptible man is to demeton is by actual human-use experience. Should the recommended treatment of apples result in a dangerous residue, it would be discovered eventually, but presumably only after some damage had been done. Because of this possibility, a group of volunteers at Wenatchee decided to eat demeton-treated fruit under controlled conditions.

It is a simple matter to draw blood for cholinesterase determination, and three is such a surplus of cholinesterase in the normal body that the individuals concerned could undergo a readily detectable reduction in enzyme level without noticeable symptoms or discomfort. Moreover, there is no evidence of any ill effects after the cholinesterase is regenerated and this replacement, even in severe depletion, takes place in a few weeks.

Fourteen volunteers each contributed three capillary blood samples on each of three successive days in order to establish a normal cholinesterase activity level for each individual. Then each volunteer ate one peach a day for 14 days. Some peeled the peaches before eating them and some did not. Ten of the group ate peaches which had been sprayed with one pint of (21%) demeton per hundred gallons 21 days before harvest and the fruit had been harvested four days before the first fruit was eaten. The other four ate untreated peaches. No individual was told which group he was in until the experiment had been completed. All participants were asked to report any illnesses or symptoms and any medications taken during the course of the experiment.

On the last day of eating peaches another series of three capillary blood samples was taken, and repeated the next two days. One week later, the same groups began eating two apples a day. In this instance, the treated apples had been sprayed with one pint of 21% demeton per hundred gallons three weeks before harvest but the fruit was harvested eight days before the first apples were eaten. None of

Table 1

Average blood cholinesterase activity values of individuals before and after eating demeton-treated fruit. One peach a day was eaten for two weeks followed by two apples a day for another two weeks. Each value is the average of nine determinations.

Group	Case No.	RBC Before Fruit	RBC After Peaches	RBC After Apples	Plasma Before Fruit	Plasma After Peaches	Plasma After Apples
Demeton-treated	1	.81	.89	.84	.77	.77	.79
	2	.85	.85	.83	.94	1.01	.98
	4	.77	.85	.81	1.18	1.19	1.15
	5	.74	.81	.81	.75	.75	.75
	7	.96	1.03	.96	1.04	1.05	.91
	8	.93	.82	.76	.76	.74	.74
	10	.89	.93	.86	1.12	1.07	1.10
	11	.79	.84	.82	.83	.83	.79
	12	.97	.91	.93	.81	.80	.78
	13	.95	.94	.91	.83	.87	.87
	Ave.	.87	.89	.85	.90	.91	.89
Untreated Controls	3	.75	.76	.73	.77	.91	.84
	6	.84	.85	.85	.85	.84	.76
	9	.87	.87	.84	.95	.93	.91
	15	.85	.79	.80	.71	.71	.70
	Ave.	.83	.82	.80	.82	.85	.80

the participants peeled the apples before eating them but some of the fruit was washed or wiped. On the 14th day of eating apples, another three-day series of blood sampling was begun.

The average cholinesterase activities of the red blood cells and of the plasma for each individual before and after eating the fruit is shown in Table 1. It is apparent that there was no significant drop in either plasma or red cell cholinesterase activity. Neither were there any complaints except for occasional mild diarrhea or constipation which occurred in both control and treated groups.

Since there was no evidence of ill effect resulting from eating one treated peach a day, it was decided to let those who wished to do so, eat as many peaches as they could in one day. This was done immediately following the two weeks diet of one peach a day. During the day following the second blood sampling, 12 of the participants ate from 3 to 10 peaches each. A comparison of the results of the blood samples drawn on the third day as compared with the first two is shown in Table 2. Eight of the group had engorged on treated peaches, four on untreated peaches and two ate none at all. Though reductions were markedly more frequent in the test group than were increases, nevertheless the changes were so small that the average showed essentially no difference.

To check these results, three of the participants who had engorged on treated fruit volunteered to repeat the process. Four days later these three had blood samples drawn at 9:00 a. m. and during the next seven hours they each ate 12 or 13 treated peaches. Following consumption of the last peach they contributed blood samples at 4:00 p. m. and again at 7, 18, 24, and 43 hours, and 3, 4, and 7 days later. Though there was some suggestion of a slight immediate rise followed by a slight drop in this series of samples, nevertheless these changes lost any semblance of significance when compared with rises that occurred in these individuals during the previous three weeks (Table 3).

Finally, following the two weeks of eating two apples a day, two of the participants engorged themselves on treated apples, again providing blood samples immediately before and at 0, 6, and 18 hours after eating 8 apples each. In this case there was another slight rise in cholinesterase values but the subsequent pattern was not consistent (Table 4).

To determine the residue present in the consumed fruit, three different laboratories were provided with similar samples composed of slices of the fruit eaten and each laboratory analyzed the fruit by the **in vitro** bio-

Table 2

Average cholinesterase activity values of individuals before and after engorgement on demeton-treated peaches.[b]

		RBC				Plasma			
			Engorgement				Engorgement		
Group	Case No.	Before Peaches	Before[a]	After	Diff.	Before Peaches	Before[a]	After	Diff.
Demeton-treated	1	.81	.89	.88	—.01	.77	.78	.77	—.01
	2	.85	.86	.83	—.03	.94	1.00	1.03	+.03
	5	.74	.80	.79	—.01	.76	.77	.73	—.04
	7	.97	1.04	1.03	—.01	1.04	1.06	1.03	—.03
	8	.74	.82	.81	—.01	.76	.74	.76	+.02
	10	.90	.88	1.02	+.14	1.12	1.08	1.08	.06
	11	.80	.84	.83	—.01	.83	.84	.81	—.03
	12	.97	.92	.89	—.03	.81	.80	.79	—.01
	Ave.	.85	.88	.89	+.01	.88	.88	.88	.00
Untreated or Unengorged	3	.88	.75	.79	+.04	.75	.92	.89	—.03
	6	.84	.87	.81	—.06	.85	.82	.88	+.06
	9	.86	.88	.85	—.03	.96	.91	.96	+.05
	15	.85	.79	.80	+.01	.71	.72	.68	—.04
	14	.83	.89	.86	—.03	1.24	1.17	1.18	+.01
	4	.77	.84	.88	+.04	1.18	1.15	1.27	+.12
	13	.95	.95	.94	—.01	.83	.87	.85	—.02
	Ave.	.83	.85	.85	.00	.93	.94	.96	+.02

[a] Before engorgement but after one peach a day for two weeks.

[b] Figures for "before peaches" are based on nine determinations: those before engorgement on six and those after engorgement on three determinations.

Table 3

Cholinesterase activity values before and at indicated times after eating a large volume of demeton-treated peaches. The weight of peaches eaten in a seven-hour period and the normal cholinesterase values six weeks earlier are also shown. Except for the normal values, all others are averages of three determinations.

	Case No.	Wt. of Peaches Eaten	Pre-Eating Normal	Pre-Engor. 9 a.m.	Post-engorgement 0 hrs.	6 hrs.	18 hrs.	24 hrs.	43 hrs.	3 days	4 days	7 days
R.B.C.	1	2044	0.81	0.93	0.90	0.95	0.87	0.88	0.81	0.86	0.92	0.85
	2	2506	0.85	0.80	0.91	0.94	0.83	0.95	0.94	0.83	0.84	0.88
	5	2410	0.74	0.86	0.88	0.87	0.79	—	0.87	0.84	0.84	0.87
	Ave.	2321	0.80	0.89	0.90	0.92	0.83	0.92	0.87	0.85	0.87	0.87
Plasma	1	—	0.77	0.81	0.84	0.80	0.80	0.79	0.76	0.76	0.79	0.88
	2	—	0.94	1.05	1.10	1.02	1.04	0.99	1.02	0.98	0.95	1.14
	5	—	0.75	0.79	0.85	0.75	0.78	—	0.75	0.72	0.81	0.78
	Ave.		0.82	0.88	0.93	0.87	0.87	0.89	0.84	0.82	0.85	0.93

Table 4

Cholinesterase activity values before and at indicated times after eating a large volume of demeton-treated apples. The weight of apples eaten in a seven-hour period and the normal cholinesterase values six weeks earlier are also shown. Excpt for the normal values, all others are averages of three determinations.

	Case No.	Wt. of Apples Eaten	Pre-Eating Normal	Pre-engorgement 3 a.m.	Post-engorgement 0 hrs.	6 hrs.	18 hrs.
R.B.C.	1	980	0.81	0.89	0.99	0.97	1.00
	2	1365	0.85	0.86	0.90	0.91	0.93
	Ave.	1167	0.83	0.88	0.95	0.94	0.96
Plasma	1	—	0.77	0.81	0.85	0.77	0.78
	2	—	0.94	1.03	1.11	1.07	1.07
	Ave.	—	0.86	0.92	0.98	0.92	0.92

chemical method described. The three laboratories agreed reasonably well in their results and showed an average of 0.3 ppm of demeton in the treated apples and 0.5 ppm of demeton in the treated peaches.

Based on the average daily consumption for 14 days of 102[1] grams of peaches and 345[1] grams of apples per person, the demeton equivalents consumed were about .05 mg. in peaches and .10 mg. in apples. The average body weight for the group was 77 kilograms. This indicates a daily dosage of about .0006 mg./kg. for the peaches and about .0013 mg./kg. for the apples. These dosages produced no apparent reduction in cholinesterase activity and no symptoms attributable to the demeton. Under conditions of engorgement, the maximum single dosage amounted to .016 mg./kg. over a period of seven hours while engorging on peaches and .004 mg./kg. while engorging on apples. Again such single dosages produced no apparent reduction in cholinesterase activity.

It is concluded therefore that normally healthy adults can consume one peach or apple a day treated with demeton according to recommendations, without producing any apparent effects.

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[1] These weights include gross weight of fruits eaten including seed, core, stems, peel, and aliquants for chemical analysis. Aliquants were about one-quarter of the weight of peaches and about one-third the weight of the apples.

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[illegible] PROGRAM
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Present Status of Our Knowledge of DDT Intoxication

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Baseless rumors of the hidden dangers of DDT—which are on a par with stories of the baneful effects of the use of aluminum cooking utensils—are obviously difficult to combat convincingly. The truth about the chance of DDT poisoning is here—it may prove helpful in answering inquiries.

The properties of DDT have been under investigation since 1939 and in this country since 1942. The compound has been in general use in this country since 1946 and previously was the subject of extensive military experience. The reasons for the continued interest in the toxicology and pharmacology of the compound lie in the fact that a greater tonnage of it is produced than any other insecticide, that it occurs as residues in food, and that it is stored in human tissue. Compared with other chlorinated hydrocarbon insecticides DDT is especially favorable for investigation, because more is already known about it and because the methods for its chemical analysis are advanced. Within limits, evaluation of related materials is bound to be influenced by what is known of DDT.

It is the purpose of this paper to review the outstanding features of our knowledge of the effect of DDT on man. Only the more important references will be given, because a far more comprehensive review should soon be available.[24]

Uncomplicated, acute DDT poisoning in man is a well described clinical entity.[19, 27, 29, 34, 39, 41, 53, 54] The onset depends on the dose; it may be as little as 30 minutes (after a dose of 20 gm.), but is usually two to three hours and may be longer. It is characterized by paresthesia of the tongue, lip, and part of the face. In more severe poisoning the paresthesia may also be detected in the extremities. The patient soon suffers from a sense of apprehension, a disturbance of equilibrium, dizziness, confusion, and—most characteristic—tremor. In severe poisoning convulsions may intervene and there may be paresis of the hands. General symptoms include malaise, headache, and fatigue. Very large doses are followed promptly by vomiting. Delayed vomiting or diarrhea, or both, may also follow. Careful medical examination during the period of severe symptoms indicates that the pupils are dilated. Except in severe poisoning the pupils react normally to light and accommodation and the eyes show no nystagmus. Sensitivity to touch and pain are exaggerated in the areas in which the patient feels paresthesia, and proprioception and vibratory sensation may be lost in the fingers and toes, but not in the more proximal joints. Tests to demonstrate coordination are performed poorly, but reflexes are normal except when the dosage has been very large. There is a slight tendency to leukocytosis without a constant deviation in the differential count, representing a nonspecific defense mechanism. A slight decrease in hemoglobin may be seen. The pulse may be quickened in mild poisoning, but irregular or abnormally slowed (45-60/min.), or both, in severe poisoning. Blood pressure, temperature, sedimentation rate, and urine remain essentially normal.

Except in the most serious cases recovery has always been well advanced or complete in 24 hours. Three persons who were estimated to have eaten 20 gm. of DDT each still showed a residual weakness of the hands after five weeks. There is no well described case of fatal, uncomplicated DDT poisoning. In one instance death followed the taking of an unstated amount of DDT powder with suicidal intent,[14] but the possible involvement of other poisons was not excluded.

In addition to the clear-cut picture of poisoning already described, there are records of dermatitis,[25, 26, 32, 35, 45, 52] purpura,[12, 30] agranulocytosis,[57] and other conditions[14, 56] representing hypersensitivity. In the few cases that have been described, the patient's exposure was not great, and in no single instance was there completely convincing evidence that the disease was in fact caused by exposure to DDT or even exposure to the formulation in which the DDT was used. This does not deny that DDT seemed to be the most likely cause in some of the reported cases. Because for nearly every known material a few people may be found who are hypersensitive, it would be unreasonable to suppose that a few people are not hypersensitive to DDT. The important fact is that the total number of reported cases is very small, particularly when one considers the tremendous amount of the compound which has been used.

The greater proportion of dermatitis cases associated with DDT have been thought to be associated with a direct action of the compound on the skin. However, even some cases of dermatitis, like other forms of hypersensitivity, have been thought to be associated with absorbed DDT. The route of exposure in these cases has generally been in doubt. It is well established from animal experiments that oily solutions of DDT are absorbed from the skin and it is also known that particles of appropriate size may enter the lung and there be absorbed. The fact remains that the well established syndrome of systemic poisoning in man has never been reported following dermal or respiratory exposure. This is true, although many persons either under the conditions of experiments, or under conditions of actual use, have received very extensive dermal[10, 13, 15, 58] and respiratory[16, 43] exposure.

Dermatitis resulting from direct exposure to kerosene and other insecticide solvents is a well recognized condition. A number of deaths have been reported following the ingestion of DDT solutions. In these instances the clinical picture has frequently been characteristic of solvent poisoning. It is apparent from animal experiments that solvents, particularly animal and vegetable oils which are digestible, enhance the toxicity of large doses of DDT. Indigestible oils may even reduce the toxicity of DDT by preventing its absorption and by their laxative action. Kerosene, which is the most common solvent for DDT, has a considerable toxicity for man,[9, 33, 46] a fact which is apparently not fully appreciated by many people. It is obvious that it is very dangerous to drink DDT solutions, but it is not possible to learn very much about the toxicity of the compound as such from a study of cases involving this kind of exposure.

No instance of chronic DDT poisoning in man has been confirmed. This is probably to be expected from what is known of poisoning in animals. Animals

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may be poisoned by moderate repeated doses of DDT, but the illness which they develop is strikingly similar to the illness which they show following a single massive dose of the compound. If dosage is continued, then the illness may be continued but, strangely enough, signs of poisoning may diminish or disappear altogether, even in the face of continued dosage. Animals appear to develop a slight but detectable tolerance. Furthermore, in view of what is known of storage, one is forced to conclude that illness related to the compound is never detectable in animals which do not at the time contain DDT. This relationship is also true of the morphological changes which have been demonstrated in the tissues and particularly the liver. Therefore, the question may be raised whether chronic poisoning has ever been demonstrated in animals. It is more nearly accurate to say that animals may continue to show illness during continued heavy exposure.

Needless to say, the repeated suggestion that DDT is the direct cause of a virus-like disease and of a psychoneurotic syndrome and is a contributing cause of poliomyelitis, hepatitis, cardiovascular disease, and cancer,[3-8, 37, 38, 40] as well as of a formidable array of animal diseases, finds no support in animal experiments or in human morbidity or mortality statistics. Fowler's study [18] in a community where suggestions of this sort had been championed failed to find any substantiating evidence, although case reports had been actively solicited from local physicians and others. Fowler did, of course, find instances of insecticide poisoning based on excessive and generally very careless occupational exposure.

The oral dosage of DDT necessary to produce illness in man has become rather accurately known. A single ingestion of 10 mg./kg. produces illness in some [27, 53] but not all [27, 44] subjects, even though no vomiting occurs. Smaller dosages generally produce no illness, although a dosage of 6 mg./kg. produced perspiration, headache, and nausea in a man who was already sickly and who was hungry at the time of eating the compound.[27] Those who have shown illness following ingestion of 10 mg./kg. have not had convulsions, but convulsions have frequently occurred when the dosage level was 16 or greater.[27] Dosages at least as high as 285 mg./kg. have been taken without fatal result.[19] However, these and even smaller dosage levels lead to prompt vomiting, so that the amount actually retained cannot be accurately determined.

The least dosage which, when repeated daily, will lead to illness in man is not known. Experiments with the most susceptible animals would lead one to expect that some individuals might show mild illness at a dosage ranging from 2.5 to 5 mg./kg./day. Although dogs withstand 10 mg./kg./day for years without any adverse effect, the fact that some human subjects are made ill by a single dose at this rate shows that man is more susceptible to DDT than the dog. It is known, as the result of an experiment now under way with human volunteers, that dosages as high as 0.5 mg./kg./day may be taken daily for several months without producing any adverse effect which the subject can detect, or which can be discovered by physical or laboratory examination. It is interesting to observe that this dosage is greater than the dosage obtained by rats whose total diet contains DDT at a concentration of 5 ppm. On such a diet adult male and female rats receive an average dosage of 0.22 and 0.26 mg./kg./day, respectively.

No quantitative estimate can be given of the dermal toxicity of DDT to man but, as already noted, dermal exposure under conditions of actual use has not been sufficient to produce systemic poisoning. In most instances authors have found that the dermal LD_{50} of dissolved DDT is somewhat greater than the oral LD_{50} of the dissolved compound for a number of species. There is complete agreement that the dermal LD_{50} of undissolved DDT is extremely high (400,000 mg./kg., or greater), and many authors have found it impossible to determine the LD_{50} under these conditions because the material was simply not toxic enough.

In a fatal case alleged to represent uncomplicated DDT poisoning, autopsy revealed moderate congestion of the lungs and severe congestion of the gastric mucosa in the region of the cardiac antrum. Animals killed by a single large dose frequently show generalized congestion, foci of hemorrhage, or pulmonary edema associated with violent convulsions or the agonal state. Animals which survive long enough to develop changes may show small areas of necrosis in the liver and, to a lesser extent, in other organs. Repeated dosages which fail to produce illness do not generally produce any change in the tissues. Relatively large repeated doses tend to produce the same tissue changes as a single large dose in most species of animals and, in fact, with the repeated doses the changes occur more regularly and tend to be more pronounced because the animals survive long enough for the lesions to mature.

Much has been made of the changes in the liver of rats maintained on diets containing as little as 5 ppm of DDT. A very complete reinvestigation of the subject by Ortega of this laboratory makes it impossible to agree with Cameron and Cheng [11] that the changes are not real but represent artifacts. The changes are very real and not only can be demonstrated by careful histological methods, but also can be observed with the phase microscope in living liver cells. Although the reality of the changes in the liver of rats maintained on relatively small doses of DDT can no longer be questioned, the significance of the changes remains a matter of considerable doubt. In the first place, these changes are considered not to be a stage in the development of the necrosis caused by larger doses, but to be an unrelated phenomenon. The minor changes under discussion are reversible. They are restricted to rodents and are especially characteristic of rats. Even in rats they do not correlate well with toxicity, for they are much more prominent in the male, whereas the female is slightly more susceptible to DDT poisoning and stores far more of the compound.

Although the liver is the organ showing the greatest histological change in animals poisoned by DDT, animals or human beings made sick by the compound suffer from dysfunction of the neuromuscular system and not from liver dysfunction. Although very large doses undoubtedly produce liver injury, the injury is frequently not sufficient to be detectable by liver function tests, nor sufficient to interfere with the animal's health. Because of the predominance of the neuromuscular disturbance, it is remarkable that competent pathologists have failed to find any extensive changes in the nervous systems of DDT-poisoned animals. In the most thorough study which has been made of the subject, Globus [21, 22] failed to find any pathology resulting from DDT in the brain or spinal cord of monkeys, dogs, cats, and rats.

DDT is stored in the adipose tissue of all mammals and birds that have been studied in this regard. Rats fed DDT daily at a constant rate store more and more of the material in their fat for a time, but gradually reach a plateau so that no additional storage occurs in spite of continued intake. The plateau or equilibrium level is characteristic of the dosage. If the dosage is doubled, then the equilibrium storage will be almost but not quite doubled. Rats and monkeys reach equilibrium in DDT storage in something less than six months. Although other animals have been studied in less detail, it appears that they store DDT in the same way. Thus, DDT storage is proportional to dosage for animals of the same sex and species. In rats there is a very distinct difference in DDT storage between the sexes; in monkeys and pigs the difference is extremely small, if a difference exists. Broadly speaking, different species of mammals and birds which have been studied store DDT at the same rate. This does not imply that species differences cannot be detected, but rather that they are small. Information so far available on the storage of DDT in man indicates that man represents no radical exception to the general rule.

DDT is broken down by the body and two of the products—DDA and DDE—have been identified. Both of these materials are formed by man. DDA, the acetic acid derivative of DDT, is excreted in the urine,[44] and DDE, the dehydrochlorinated derivative, is stored in the fat.[30, 47] Although less than half of the DDT-derived material in the general diet is DDE, more than half of such material stored in human fat tissue is DDE. This indicates that human beings convert DDT to DDE. Final evidence for the conversion was provided by a study of persons exposed to relatively large doses of *p-p'*-isomer.

DDT-derived material is excreted in man in urine, feces, and milk. Animal studies have indicated that DDT occurs in the secretion of the skin and that it passes the placental barrier into the fetus.

The absorption and subsequent excretion of chemical substances is more general than some suppose. Thus, Sapeika [48] has recorded the presence of alcohol, caffeine (or a derivative), nicotine, and a wide range of drugs in human milk. The excretion of a small amount of lead must be considered normal; its concentration in the urine is usually at a rate of 0.005–0.05 ppm, and may average 0.1 ppm in areas where there is increased environmental exposure.[42] It is only when higher dosage levels and thus higher excretion levels are encountered that there begins to be a danger of lead poisoning. In a similar way, the mere presence of stored material in the tissues does not necessarily represent an injury, and when injury is involved it may not correlate with the degree of storage.[17, 31]

One of the most promising recent developments is the demonstration that exposure of human beings to significant dosage levels of DDT can be quantitatively related to the excretion of DDA in the urine. Formerly, the measurement of DDA in the urine has been accomplished or considered only in connection with single doses that were at or extremely near the toxic level. It has been a great drawback in the past that the only objective measure of human exposure to DDT was storage of the material and its derivative, DDE, in the fat. Persons who had extensive occupational exposure were frequently and understandably unwilling to submit to biopsy because they were healthy and had nothing but inconvenience to gain from volunteering. It should now be possible to measure objectively the exposure of workers, some of whom have been constantly engaged in the manufacture or formulation of DDT for eight years or more.

Probably the most important need in the field of DDT research at this time is a complete balance study. It is true that Judah [28] was able to account for essentially all the DDT injected intravenously into rats three hours before sampling. However, the conditions of the experiment were highly artificial, and success has been very much poorer in accounting for the total dosage under more practical conditions. Considerable progress has been made recently, and it appears possible that the problem will be solved within the next few years. Until a balance study has been completed under conditions reasonably similar to those encountered by human beings, there will remain a question whether the portion of the dosage that cannot now be accounted for has some significance which we do not now grasp.

The fact that research remains to be done is not a valid reason for alarm. A great deal is known about the toxicology and pharmacology of DDT, but its basic mode of action remains unknown. The same is true of aspirin and barbiturates which are the cause of many more fatal accidents than all the insecticides taken together.

In evaluating the safety of DDT one must observe that there has been a very small number of accidents with it in spite of the tremendous tonnage that has been used. Careful studies of workers with extensive occupational exposure have indicated the safety of the material[1, 2, 18, 20, 23, 40, 50, 51] and it is clear that more occupational disease has been caused by the solvents in DDT formulations than by the compound itself. It has even been suggested that more people are injured by spray rigs and other devices for distributing insecticides than by all insecticides themselves.

DDT Residues—Because of the chemical stability of DDT under field conditions, and because the compound is stored in fat, it was realized at least as early as 1945 that DDT presents a potential residue problem. There has been much discussion of this problem and much investigation of certain aspects of it. Most of the published values for residues concern only a single food product and frequently concern products that were treated under artificial conditions and at rates higher than those recommended by agricultural authorities and followed in actual practice. Until recently, there was a strange lack of information on the amount of DDT in food which might reasonably be expected to form the total diet. Walker, et al.,[53] studied restaurant meals and also meals in an institution. A number of restaurants were investigated. An effort was made to choose the most popular breakfast, lunch, and supper offered each day by each restaurant. Extremely expensive and specialty items were avoided. In each instance a complete balanced meal was analyzed. No meal was found, either in the restaurants or in the institution, which failed to contain some DDT, although some individual food items in the meals were regularly free of the compound. Fatty foods, or foods cooked in fat, tended to contain more DDT than other foods. The average concentration of DDT in the food on a dry basis was 0.31 ppm. The average total amount for breakfast, plus lunch, plus supper, was 184 μg., which would determine for a man of average size a dosage of 0.0026 mg./kg./day. This dosage is to be compared with a dosage of 0.5 which has been taken without injury by volunteers.

In conclusion, there is abundant evidence to indicate that DDT is a safe material under actual conditions of use. Although it occurs in our diet, and is stored in our fat, there is no indication that the amounts involved are injurious. Human beings, as well as animals, can withstand much greater exposure and much greater storage than are now prevalent. The greatest recent advances in our knowledge of DDT are those which define the current exposure and the magnitude of tolerable dosages involving this compound which has contributed so significantly to public health and to agriculture. This quantitative information based on human rather than animal exposure should do much to satisfy any reasonable doubts about the safety of DDT.

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SERUM ORGANOCHLORINE PESTICIDES IN MENTALLY RETARDED PATIENTS ON DIFFERING DRUG REGIMENS

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Serum organochlorine pesticides in mentally retarded patients on differing drug regimens

Serum concentrations of p,p'DDT and its metabolite p,p'DDE were estimated by electron-capture gas chromatography in a group of mentally retarded institutionalized patients receiving antiseizure and tranquilizing chemotherapy. Control patients not taking drugs tended to resemble the general population in terms of organochlorine residues, but drug takers showed greatly lowered levels of both p,p'DDE and p,p'DDT. The combination of phenobarbital and diphenylhydantoin seemed to be the most effective in depressing serum pesticides and remained so even in patients taking other drugs concurrently. p,p'DDE was found at detectable levels in all patients sampled, but the frequency of occurrence of p,p'DDT was considerably decreased among the drug takers. This was particularly true of patients receiving both phenobarbital and diphenylhydantoin concurrently. The possibility that the catabolism of p,p'DDT is induced preferentially to that of p,p'DDE by the phenobarbital-diphenylhydantoin combination is discussed.

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The biological persistence of organochlorine insecticide residues, which include dichlorodiphenyltrichloroethane (DDT) and its various congeners, has become a topic of wide-spread concern. Unlike the relatively short-lived (but usually more toxic) organophosphate and carbamate pesticides, members of the organochlorine series are capable of being stored and concentrated in the adipose tissue of organisms for long periods of time. This concept of protracted residue storage within the biota poses a problem of increasing importance to investigators.

Drugs, pesticides, and other pharmacologically active substances are metabolized in the body chiefly by nicotinamide (NADPH)-dependent hepatic microsomal enzyme systems.[7] Although relatively little is known of the specific biochemical mechanisms involved, it appears that cytochrome P-450 has a major role in the hepatic detoxification of many substances.[6, 8] This hemoprotein, thought to be the terminal oxidase, has been found in animal experiments to increase following treatment with various drugs and organochlorine insecticides.[11]

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Table I. *Characteristics of each group of patients sampled*

Group	Drug regimen	No.	Male	Female	Average age	Average tenure in institution	Patients with differing dietary status	
							Low-calorie	Low-sodium
Control								
A	None	46	27	19	36	17	8	—
Drug								
B	Phenobarbital	8	2	6	32	19	1 (Diabetic)	—
C	Diphenylhydantoin	5	2	3	32	22	—	—
D	Phenobarbital and diphenylhydantoin	15	8	7	38	28	2 (1 Diabetic)	—
E	Phenobarbital, tranquilizers, and sedatives	18	7	11	31	16	3	—
F	Diphenylhydantoin, tranquilizers, and sedatives	19	15	4	29	16	1	1
G	Phenobarbital, diphenylhydantoin, tranquilizers, and sedatives	24	14	10	33	18	4	1
H	Tranquilizers and sedatives	28	21	7	25	15	5	—
B-H	All drug classes combined	117	69	48	31	18	16 (2 Diabetic)	2

Recent studies have shown that certain drugs and chemicals are capable of increasing microsomal enzyme activity—a process known as enzyme induction. Some of the numerous and chemically diverse substances which are capable of inducing the catabolism of one another include various organochlorine insecticides (DDT, dieldrin, chlordane), anticonvulsants (phenobarbital, diphenylhydantoin), antipsychotic tranquilizers (chlorpromazine, trifluoperazine), general tranquilizers and sedatives (meprobamate, chlordiazepoxide), etc.[1] For a more comprehensive analysis of enzyme-inducing substances, the reader is referred to the excellent reviews on this subject by Conney,[1] Fouts,[6] and Kuntzman.[8]

Understandably, such findings have tended to emphasize the importance of drug-pesticide interaction, and especially the intriguing possibility of reducing body burden by enzyme induction. Chemotherapeutic depletion of organochlorine residues has recently attracted the attention of numerous investigators. Davies and associates[3, 5] have reported greatly decreased serum levels of 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (p,p'DDE) in a group of mentally retarded persons being treated with the anticonvulsant drugs phenobarbital and diphenylhydantoin. Conversely, the presumed ability of DDT to induce the degradation of phenobarbital has been utilized medically by Rappolt[12] who has reported successful treatment of barbiturate overdose with large oral doses of DDT.

As a corollary to these and other findings, we have examined serum organo-

Table II

Group	*Drug regimen*	*No.*
A	Control patients taking no drugs of any kind	46
B	Patients taking phenobarbital only	8
C	Patients taking diphenylhydantoin only	5
D	Patients taking both phenobarbital and diphenylhydantoin in combination	15
E	Patients taking phenobarbital in combination with tranquilizers and sedatives[*]	18
F	Patients taking diphenylhydantoin in combination with tranquilizers and sedatives	19
G	Patients taking phenobarbital and diphenylhydantoin in combination with tranquilizers and sedatives	24
H	Patients taking tranquilizers and sedatives only	28

[*] It was initially hoped that in addition to patients taking phenobarbital and diphenylhydantoin, sufficient numbers of individuals taking other drugs exclusively could be obtained for inclusion in this study. Unfortunately, since the number of patients taking only one other drug in common was quite small, it was necessary to consider them together as a single group taking "tranquilizers and sedatives." Patients so designated were those who had been receiving any of the following drugs: phenothiazine tranquilizers—chlorpromazine, promazine HCl, trifluoperazine, thioridazine; various other classes of tranquilizers and sedatives—chloral hydrate, meprobamate, chlordiazepoxide, diazepam, hydroxyzine HCl, reserpine.

chlorine residues in a group of mentally retarded institutionalized patients, 117 of whom had been receiving large doses of various combinations of anticonvulsants, tranquilizers, and sedatives for lengthy periods of time.

Methods

Selection of patients. A group of 163 (96 men, 67 women) mentally retarded patients at the Idaho State School and Hospital, Nampa, Idaho were sampled. During the sampling period of 9 months, a 10 ml. post-prandial whole blood sample was obtained from each patient at 3 month intervals (three samples from each patient). All analytic results for each individual were then based on the average values taken from the three samples. Ages of the patients ranged from 9 to 88 years (median 33). Tenure within the institution varied from one to 49 years (mean 16). For the most part, diets were identical, consisting of food obtained and prepared by the institution. Exceptions to this, as shown in Table I, included 24 patients (2 of whom were diabetic) restricted to 1,200 calories per day, and 2 patients on a low-sodium diet. Retardation etiologies (when known) within the group represented a wide spectrum of causative factors. All patients were Caucasian with the exception of one Bannock-Shosone Indian and one Nez Perce Indian.

Patients were selected purely on the basis of their drug regimens, freedom from cachexia, and suitability for venipuncture. All persons chosen had received continuous doses of their respective drugs for a minimum period of 3 months prior to the onset of sampling.

Due to the complexity of the differing drug regimens and doses involved, the scheme of patient classification shown in Table II was adopted.

No drugs not listed in Table II were being taken by any of the patients. Additional characteristics of the patient groups are summarized in Table I.

Extraction. Following whole blood centrifugation, the serums were extracted for organochlorine pesticides by means of a semiautomated, modification of the hexane procedure of Dale and associates.[2] Serum, 2 ml., was combined in a round-bottom tube with 6 ml. of nanograde hexane. This mixture was then gently agitated for 2 hours on a slow-speed (50 to 60 r.p.m.) rotating mixer. The hexane layer, 5 ml., was then transferred to a 10 ml. concentrator tube by pipette and evaporated on a steam bath to a final volume of 1 ml. by means of a two-ball Snyder column. Portions, 5 μl, were then injected into a

Micro-Tek 220 gas chromatograph equipped with two differing columns and tritium foil electron-capture detectors. This dual-column technique was used in order to provide reliable confirmatory analysis of the samples tested.

Analysis. The operating parameters for gas chromatographic analysis were as follows: Columns—4 per cent SE-30, 6 per cent QF-1 on Chromosorb W, H.P., 80/100 mesh and 1.5 per cent OV-17, 1.95 per cent QF-1 on Chromosorb W, H.P., 100/120 mesh; Temperatures—columns 200° C., injection chamber 220° C., detector 205° C.; Column gas flow—SE-30, QF-1, 90 ml. per minute and OV-17, QF-1 70 ml. per minute; Approximate elution times (p,p′DDT)—SE-30, QF-1, 20 minutes and OV-17, QF-1 19 minutes.

Both columns were 6 feet in length and made of borosilicate glass (Corning No. 237300) having an inside diameter of 5/32 inch and an outside diameter of 1/4 inch. The OV-17 column was prepared using a solvent/support ratio of 95/20 (milliliters per gram) and placed in a column conditioner for 24 hours at 245° C. The SE-30 column solvent/support ratio was 100/20 and pre-conditioning was at 60 hours at 245° C. Liquid phases and solid support were obtained from Supelco, Inc. Detector responses were linear for all the serum pesticide concentrations analyzed.

Nitrogen was the carrier gas used in all instances. Quantitation was based on relative peak heights, and qualitative retention times were based on the retention time of aldrin (Octalene). Per cent recovery ranged from 80 to 100 per cent and was based on the prior addition of a known amount of aldrin to each serum sample as an internal standard. Although traces of several different serum pesticides were detected in some cases, this report will deal only with 1,1,1—trichloro-2,2-bis(p-chlorophenyl)ethane (p,p′-DDT) and its metabolite, p,p′DDE.

Results

Results of the serum analyses are shown in Table III. Sera of all of the patients investigated, regardless of etiology and drug regimen, were found to contain detectable levels of p,p′DDE in at least one of the three consecutive serum samples obtained. The 46 persons taking no drugs and serving as controls (Group A) had a mean serum p,p′DDE level of 31.2 parts per billion (p.p.b.), while p,p′DDT averaged 5.1 p.p.b. In contrast to the control group, patients receiving chemotherapy (Groups B through H taken as a whole) had much lower mean serum levels (7.4 p.p.b. p,p′DDE and 1.1 p.p.b. p,p′DDT), as well as a much lower frequency of p,p′DDT occurrence.

The 8 patients taking only phenobarbital (Group B) and the 5 taking only diphenylhydantoin (Group C) had similar mean p,p′DDE levels (6.8 and 5.3 p.p.b., respectively) and ranges (Table III). The same was true for p,p′DDT, except that this residue was found in only 62.5 per cent of the samples from people taking only phenobarbital, in contrast with the 80 per cent frequency of occurrence noted in patients taking only diphenylhydantoin.

Group D, comprised of 15 patients taking both phenobarbital and diphenylhydantoin in combination, had the lowest means, ranges, and standard deviations for serum p,p′DDE and p,p′DDT. Although all patients receiving this particular drug combination showed detectable levels of p,p′DDE in at least one of the three samples taken, the mean for p,p′DDE of this group was only 1.6 p.p.b. and the range was only 0.1 to 2.9 p.p.b. In Group D, p,p′DDT was detected in only 40 per cent of the samples with a mean level of 0.1 p.p.b. and a range of 0 to 0.5 p.p.b.

Patients receiving phenobarbital and tranquilizers and sedatives (Group E) resembled those taking diphenylhydantoin and tranquilizers and sedatives (Group F) with respect to p,p′DDT. The means, per cent occurrences, ranges, and standard deviations for this residue were quite similar in both groups, as shown in Table III. Mean levels of p,p′DDE, however, were found to differ somewhat, with 7.8 p.p.b.

Table III. *Comparison of serum p,p′DDE and p,p′DDT in the different patient groups sampled*

			*Serum pesticides (parts per billion)**							
			p,p′DDE				*p,p′DDT*			
Group	*Drug regimen*	*No.*	*Per cent occurrence*	*Mean*	*Range*	*S. D.*	*Per cent occurrence*	*Mean*	*Range*	*S. D.*
Control										
A	None	46	100	31.2	6.4- 94.7	18.6	97.8	5.1	0-13.7	2.9
Drug										
B	Phenobarbital	8	100	6.8	1.3- 16.9	5.4	62.5	0.7	0- 1.7	0.6
C	Diphenylhydantoin	5	100	5.3	1.2- 17.4	6.8	80.0	0.9	0- 1.7	0.6
D	Phenobarbital and diphenylhydantoin	15	100	1.6	0.1- 2.9	1.0	40.0	0.1	0- 0.5	0.2
E	Phenobarbital, tranquilizers, and sedatives	18	100	7.8	1.9- 25.1	7.3	50.0	1.1	0- 7.2	2.1
F	Diphenylhydantoin, tranquilizers, and sedatives	19	100	4.5	0.3- 27.4	5.9	55.6	1.3	0-10.2	2.5
G	Phenobarbital, diphenylhydantoin, tranquilizers, and sedatives	24	100	3.1	0.4- 9.7	2.2	37.5	0.5	0- 3.0	0.8
H	Tranquilizers and sedatives	28	100	16.6	1.5-106.4	21.0	71.4	2.3	0- 7.1	2.0
B-H	All drug classes combined	117	100	7.4	0.1-106.4	12.1	55.6	1.1	0-10.2	1.8

Analysis by t test found p,p′DDE and p,p′DDT levels in each group of drug takers to be significantly lower than those of the control group ($p < 0.005$).

*As based on the average of three serum analyses per patient.

for Group E and 4.5 p.p.b. for Group F.

The combination of phenobarbital and diphenylhydantoin, even when given in conjunction with various other drugs (Group G), was found to dramatically lower p,p′DDE and p,p′DDT mean levels, although not to the extent noted in those taking phenobarbital and diphenylhydantoin alone. Serum p,p′DDE in Group G patients averaged 3.1 p.p.b. and ranged from 0.4 to 9.7 p.p.b. P,P′DDT averaged only 0.5 p.p.b., ranging from 0 to 3.0 p.p.b. This group also had the lowest frequency of p,p′DDT occurrence (37.5 per cent) which is comparable to the 40 per cent occurrence noted in the Group D patients taking phenobarbital and diphenylhydantoin alone (Table III).

Patients in Group H taking miscellaneous tranquilizers and sedatives showed serum pesticide levels intermediate between those of the control subjects of Group A and the patients in Groups D and G receiving the various phenobarbital and diphenylhydantoin combinations. P,P′DDE in Group H ranged from 1.5 to 106.4 p.p.b. (the highest mean individual level of the entire study) and averaged 16.6 p.p.b. P,P′DDT averaged 2.3 p.p.b. and ranged from 0 to 7.1 p.p.b. (Table III).

Serum pesticide residues in the 26 patients restricted to low-calorie or low-sodium diets (Table I) were not appreciably different from those of the other patients receiving comparable drug regimens. Persons within each drug group who were known to share in common any inborn errors of metabolism (e.g., diabetes mellitus, phenylketonuria, the various storage diseases, etc.) were not outstanding with respect to pesticide levels, as were those with other known genetic defects.

Discussion

The 100 per cent occurrence of p,p′DDE in all the institutionalized patients, despite their drug therapy, is at variance with the findings of Davies and associates,[3,5] who reported zero p,p′DDE levels in both whole blood and surgical fat samples obtained from a group of patients receiving concurrent phenobarbital and diphenylhydantoin therapy. Zero p,p′DDE levels in adipose tissue would seem especially unlikely, since the presence of even trace amounts of p,p′DDE in serum would tend to imply that lipid storage, however minimal, could still occur. However, this discrepancy might also be due to our use of the average value obtained from three consecutive samples from each patient, rather than a value obtained from a single sample.

In a previous study, we[15] established base-line serum levels for p,p′DDE (22.0 p.p.b.) and p,p′DDT (4.7 p.p.b.) in a group of 1,000 Southern Idaho residents, most of whom were from the same rural county in which the institution is located (due to recent advances in extraction techniques and instrumentation, these values are somewhat low) Ninety-nine and eight-tenths per cent of these 1,000 persons had detectable levels of p,p′DDE, while 84 per cent also had detectable serum p,p′-DDT. Except for their higher mean levels of serum organochlorine residues, the institutionalized control subjects in Group A tended to resemble the general local population. The most notable difference was the higher frequency of p,p′DDT (97.8 per cent) in the Group A patients, but this is most likely due to improved methodology. Among the patients classified as drug takers, depression of serum organochlorine residues is quite obvious. Of all the patients sampled, those in Groups D and G provide the most conclusive evidence of such depletion. However, although these subjects displayed the lowest mean levels, ranges, and standard deviations for p,p′DDE, it was present in all.

Perhaps the most dramatic difference noted in the drug takers was that many of them had no detectable serum p,p′DDT. In Groups D and G, taking only phenobarbital and diphenyhydantoin, p,p′DDT was detected in only 40.0 and 37.5 per cent, respectively. Such a depletion of p,p′DDT relative to p,p′DDE seems remarkable in view of the findings in the control group and the general population.

The biodegradation of p,p′DDT is currently thought to follow the general pattern described by Peterson and Robison,[10] in which DDT from external sources is mostly converted to DDD, which is further degraded to the aldehyde form, DDA, and excreted via the urine and feces. Although small amounts of DDT may be converted to DDE, the main source of DDE is thought to be dietary.[9] DDE also appears to be much more stable within the body; the minimal biodegradation that normally does occur being probably through a different metabolic pathway.[9,13] The human dietary intake of both DDT and DDE appears to be quantitatively quite similar.[4]

The greatly decreased DDT levels in Group D and Group G patients might be explained as follows: Since most of the patients shared a common diet within the institution and since their pesticide residues did not differ greatly from those of others in their drug group who were on special diets, it is unlikely that the decreased DDT is due to differences in intake. Because both DDE and DDT levels in Groups D and G are markedly lower than those detected in the control and general population groups, it is reasonable to assume that both substances are being biodegraded at an increased rate by induced mircosomal enzyme activity. The relatively low DDT levels thus imply some sort of preferential efficacy of the phenobarbital-diphenylhydantoin combination in inducing the pathway for DDT catabolism over that of DDE. This possibility should be examined further by the montoring of urinary DDA levels, which have been shown by Roan and associates[13] to be a useful index of DDT breakdown.

The decreased serum DDE levels in the drug takers is perhaps more difficult to explain, since comparatively little is known about the metabolic breakdown of this substance. However, since some enzyme inducers do deplete DDE, it seems reasonable to assume that there is a hepatic microsomal enzyme which is specific for DDE catabolism and biochemically distinct from the pathway for DDT and that this detoxification mechanism is somehow induced into supernormal activity by both phenobarbital and diphenylhydantoin, and even more by the combination of the two.

Another factor which may contribute to serum depletion of both DDT and DDE is protein binding. Schoor[14] has suggested that some drugs may have a greater affinity for serum proteins than do the pesticide residues in question, thereby displacing them from their serum binding sites and hastening their breakdown and excretion. In the case of serum DDE, such drug displacement could conceivably lower serum levels by allowing more unbound DDE to leave the serum and become incorporated into lipid storage sites. Whatever the mechanisms involved, we have further confirmed the abilities of phenobarbital and diphenylhydantoin, and to a lesser degree mixed groups of transquilizers and sedatives, to lower serum pesticide levels.

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THE TOXICITY OF DIELDRIN TO MAN

Report on a Survey

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SYNOPSIS

Increased use of dieldrin for malaria control has been caused by several factors, including sporadic resistance of some vectors to DDT. Poisoning of spray-men by dieldrin has been recognized in five widely separated countries and reported informally from several others. In some cases illness has recurred months after the last exposure. Observations of antimalaria programmes in Kenya, Tanganyika, Indonesia, India (Bombay State), and Iran and a review of the relevant literature have shown that the hazard associated with dieldrin is proportional to the degree of workers' exposure as determined by concentration of spray, area of bare skin, duration of contact, and lack of hygiene. Measurements of workers' exposure and a review of toxicity by different routes incriminates skin contamination as the greatest hazard under practical conditions. It is considered that dieldrin should not be used without justification; if it is required, then certain individual and group protective measures listed in this paper may minimize, but not necessarily eliminate, the risk. The author enumerates certain features of the toxicology of dieldrin which require intensive research.

There has been an increase in the number of countries from which reports have been received of poisoning of workers carrying out indoor residual spraying with dieldrin in antimalaria programmes. There has also been an increase in the number of species of *Anopheles* showing resistance to DDT. In many instances, dieldrin has been the most effective insecticide available to control these resistant mosquitos. For this and other less cogent reasons, the use of dieldrin has increased in countries where workers are not accustomed to the use of toxic chemicals. The World Health Organization is actively supporting antimalaria programmes in several of these countries.

Because of these facts and because the dosage of dieldrin which spray-men receive is so intimately connected with the spraying practices which they employ, the World Health Organization sent the present author as toxicologist to observe actual programmes in Kenya, Tanganyika, Indonesia, India (Bombay State), and Iran. This report presents the observations and conclusions arising from the survey and from a review of the relevant literature.

The clinical character of dieldrin poisoning in man is well known (Blazquez & Bianchini, 1956, 1957; Hayes, 1957; Patel & Rao, 1958; see also Haworth 1)) and is touched on only incidentally in this report.

Observations

Nandi Project, Kenya

This project was carried on using dieldrin for three years (1954-56) and discontinued because malaria control was considered adequate and it was desired to follow the epidemiology without further use of insecticides. The occurrence of malaria had been epidemic. The parasite rate had risen to about 60% every 2-3 years among a population of about 80 000 people; it was supposed that each epidemic spread from hyperendemic areas on two sides of the Nandi region. However, the epidemics were never fully explained. *Anopheles gambiae* was presumably the main vector; its incidence was low during outbreaks but may have been high during the time of actual transmission. It was not fully ascertained whether biting was mainly indoor or outdoor. In any event, *A. gambiae* virtually disappeared from huts and the parasite rate fell to near zero while dieldrin was used.

The conditions under which dieldrin was used safely in the Nandi project are briefly as follows. About 40 labourers and 15 supervisory staff were employed each year for a maximum of four months in the application of 1.7% dieldrin. New workers were employed each year. The men were in the habit of washing frequently and they continued this practice when they were at work without the necessity of being constantly reminded. Soap was supplied free. In addition, a rather impressive array of protective equipment was supplied and was actually used. It is noteworthy that face shields were well accepted. Although uniforms were not supplied, the workers generally wore old clothes during spraying and changed to fresh clothing after bathing at the end of the day. It is said that a pump pressure of 50-70 pounds per square inch (p.s.i.) (or about 3.5-5 kg per cm^2) was used.

Two instances of undiagnosed chest trouble and two cases of dermatitis were noted during the first year of spraying. The possibility that these illnesses were related to dieldrin was considered, and although it could not be ruled out completely, it seems very unlikely because similar difficulties have not been prominent in other programmes that have involved serious poisoning.

One man died after ingesting an unmeasured quantity of dieldrin which he had stolen while he was employed on the Nandi project.

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[1] Haworth, J. (1955) *Observations on possible toxic effects of dieldrin on mammals* (Unpublished working document WHO/Insecticides/60)

The project did result in the death of a few cows and goats. It is thought that the animals drank dieldrin or licked up spray which had been spilled. In at least one instance the spray-men ate a goat which had just died of dieldrin poisoning; they suffered no observable effect.

Although they are not directly related to the question of the safety of dieldrin, certain other observations are of interest. A few months after the first spraying, there was a great increase in houseflies. The local people generally attributed this increase to the use of dieldrin but there is some question whether a relaxation of sanitation may not have been to blame. In any event, it is certainly a fact that lizards and some other predators of flies were killed. Dieldrin was not applied to breeding sites and it seems, therefore, that it cannot have affected the immature stages of the flies which, in fact, were observed to be unharmed. The same degree of increase in flies is said to have followed the use of lindane. The resistance of houseflies in Kenya to insecticides has been reported by McMahon (1957).

In addition to the Nandi project and Taveta-Pare project (see below), it is known that Mombasa has been partially protected from malaria by the spraying of huts in a 2-mile barrier zone around the city. The insecticide is sprayed on a short time before the rainy season and takes only a few weeks to apply.

Taveta-Pare Project, Kenya and Tanganyika

The Taveta-Pare project was established in June 1955 entirely for experimental purposes. The primary objective was a study of the general health of a population associated with the control of endemic malaria. Secondary objectives included studies of the persistence, effectiveness, and safety of dieldrin. The population among whom malaria has been controlled have been subjected to a general physical examination and their weights and haemoglobin and serum protein levels recorded. Similar examinations have been done on the spray-men at the beginning and end of each spraying cycle. The dieldrin deposits have been investigated and the application procedures regulated to produce a theoretical deposit of 0.4-0.5 g per m^2 and a measurable deposit of 0.3-0.4 g per m^2. A very detailed study of the exposure of the workers has recently been published by Fletcher et al. (1959).

Much additional information about the conditions under which dieldrin was used safely in the Taveta-Pare project has also been given by the same authors. In summary, the use of a dilute formulation (0.55%) of dieldrin and the use of acceptable, effective devices has limited the exposure of spray-men to a measured skin dose of 1.8 mg per kg of body-weight per day. This exposure is undoubtedly reduced in practice by washing with soap and water. However, it has not been possible to measure the degree of reduction. The pump pressure was 50 p.s.i. (3.5 kg per cm^2).

For the purpose of evaluating safety, the project offers the advantage that most of the men with significant exposure have been employed throughout the duration of the project, that is, for a total of four years or about 900 days of actual spraying. The only difficulty in this evaluation is that the total number of such men is small, being only 10, of whom only 8 have participated in the entire project. Statistically, it is possible that no poisoning would occur in such a small sample under conditions which would produce poisoning in as much as 10% of a large population at risk. However, the fact remains that no instance of poisoning has been revealed either through examination or through the complaints of the men. It seems likely that good morale is important in obtaining the full co-operation of spray-men in safety measures. Spray-men on the Taveta-Pare project are well paid relative to other local labour.

By direct observation, I was able to confirm that some contamination of the workers does occur, but that it is minimal. The conditions of spraying are frequently difficult: I saw a man spray an outbuilding which was so small that he had to kneel during the entire operation. At the end of the day's work the spray-men's hats and face shields were grossly wet with spray. The surfaces of the right shoulder and sleeve of the spray-men's overalls were slightly damp but the dampness did not extend through the cloth.

On one occasion a pumper was seen to pick up the small knapsack in which dieldrin powder is carried in such a way that part of his hand was coated with the powder. In another instance, soil wet from spillage was left uncovered and accessible to chickens.

Although most houses were completely cleared of furnishings, bags of rice or cereals (probably weighing about 70 kg) were sometimes left in houses during spraying because they were too heavy to move. Samples of maize and maize flour from such bags left in houses during spraying have been examined and the dieldrin content has been found to range from 0 to 2.8 parts per million.

In the Taveta-Pare area, spraying is done with stirrup pumps rather than with compression sprayers, and these pumps are thought to have the advantage of being carried in such a way that the left shoulder and back are not contaminated. I observed some contamination of the side of the right leg of men carrying stirrup pumps and related equipment; however, the contamination was not wet but dusty and, therefore, not so likely to reach the skin as the leakage from a compression sprayer.

Some chickens have been killed by dieldrin during the project. Many more were killed during the first cycle of spraying than during subsequent cycles. This is attributed to the fact that flies were not resistant to dieldrin during the first cycle so that many flies were killed and then eaten by chickens at that time.

The following facts, although not directly related to the Taveta-Pare project, are of interest. In 1956, 2% granular dieldrin was used for the control of mosquito larvae in tests at Arusha, Tanganyika; the men used gloves and masks and washed; there were no untoward effects.

Dieldrin has also been used to a very small extent at the same station for crop spraying. Dieldrin emulsion was mixed in the spray tank of the aircraft so that exposure of the applicators was minimal. Dieldrin has also been used in Uganda as a residual spray against tsetse flies. An emulsion was applied to vegetation, especially the underside of tree limbs. The work was closely supervised and resulted in no known injury.

In Kenya, dieldrin is used for locust control about four months each year (personal communication from Dr Stott, Special Medical Officer. Department of Labour, Kenya Government). Application is made by ground teams and also by aircraft using a low-volume exhaust sprayer. The formulation used is a 20% solution in kerosene. Very little of this kind of application has been made, but it is proposed to expand the work.

Indonesia

Malaria control in Indonesia is carried out by the Government of Indonesia through the Malaria Institute (Lembaga Malaria) with the technical and financial aid of the World Health Organization and the International Cooperation Administration. Representatives of the three agencies form a board which sets policy. However, execution of the work and the collection of epidemiological and entomological statistics are the responsibility of the Malaria Institute. Very important in the long chain of command are the regency doctors, who carry on private practice as well as official duties.

The project is aimed at malaria control in Java. Houses in some parts of the island have not yet been sprayed. The resistance of the chief vector (*A. sundaicus*) in some parts of the coastal area led to the substitution there of dieldrin for DDT in 1955. The substitution of dieldrin was extended to many areas beginning in 1956. The insecticide was used at a rate of 0.25 g per m^2 at first, but later at 0.50 g per m^2. Dieldrin has been used in the three provinces of Java. The substitution was not confined to areas where resistance had been demonstrated.

The number of workers who have been exposed to dieldrin is great and the amount of dieldrin issued is also great, as shown by the following figures for Central Java alone up to January 1958:

Residency	*Cases of dieldrin poisoning*	*Dieldrin issued (kg)*
Semarang	23	33 702
Pati	15	15 225
Pekalongan	1	36 055
Kedu	4	9 526
Banjumas	0	12 841
Total	43	107 349

Because of the size of the project and for other reasons it was not possible to collect information on spraying conditions in the same detail as was possible for the Taveta-Pare project, for example. Briefly, the teams which I observed using dieldrin were no more careful than the team which I saw using DDT. On one occasion, I saw a mixer scooping up dieldrin powder with his bare hands so that his hands and wrists became completely white. He rinsed the material off with water but without removing his wrist-watch. Frequent contamination of the hands with a dilute suspension was the rule rather than the exception. It was my impression that a smaller volume of spray mist bounced back on the Indonesian spray-men than on the spray-men in the Taveta-Pare area. This seems to be explained by the fact that the average Indonesian house is larger than the African hut and is frequently well ventilated either by a completely open space between the wall and the roof, or by many small holes in the matting which forms the walls, or by both. On the other hand, the Indonesian spray-men had very much more skin area exposed to spray than their African counterparts. Other differences are the greater concentration of the spray used in Indonesia and the lack of any vacation period for the Indonesian workers. The pump pressure measured in Indonesia was variable and often high.

Little or nothing is removed from Indonesian houses before they are sprayed. Matting for beds is rolled up. Some food is covered and some vessels for food are overturned so that the spray falls on the underside. However, even these precautions are not invariably taken; and a case has been reported in which the spraying of cooking utensils was followed by poisoning. Only the mother in the family ate the contaminated food and only she became sick. The character of the disease is not known but it is understood that the patient survived.

The occurrence of the 43 cases of dieldrin poisoning in Central Java mentioned earlier became generally known as the result of observations by Dr R. Hasmo Soegijarto, Malaria Advisor to the Inspector of Health for Central Java. He described 15 of the cases in some detail (personal communication, 1958). The reported signs and symptoms included headache, sweating, weakness, loss of consciousness, and convulsions. He regarded the diagnosis as tentative, largely, it seems, because many of the fits occurred when the workers were going to and from home rather than when they were in direct contact with dieldrin.

The histories of nine of these cases, whom I was able to examine myself, are given below. The case numbers correspond to those assigned by Dr Soegijarto with the exception of cases 16 and 17, on which he did not report.

Case 1. L., a 20-year-old unmarried spray-man, worked with DDT for one year and with dieldrin for one year before being affected. He wore a sports shirt with long trousers while spraying. He had no hat or shoes. He washed only his hands while at work and used no soap because none was provided. He bathed at home with his own soap but it took him about two hours to go home after work and he bathed only after eating and resting. He was aware that spray fell on him while he worked. He worked 25-26 days per month, including half a day on Friday. He had Sundays off.

In August 1957 he was hospitalized for 15 days for typhoid fever and he rested for a month after leaving the hospital. Shortly after returning to dieldrin spraying he suffered a fit. At that time he complained of weakness, headache lasting about an hour, and nausea, which was present before as well as after the fit. He was unconscious for about half an hour. The relationship of the first and subsequent fits to dieldrin exposure was as follows:

11 September 1957	Continued dieldrin exposure
22 September 1957	
4 January 1958	
4 February 1958	
1 June 1958	Office work during March and April and DDT spraying during May and June

The last fit, which lasted about five minutes, was observed by the spray-man's brother, who was a supervisor of the squad. There was no personal or family history of convulsions. The man stated that he felt well and that he had grown bigger and stronger while working as a spray-man. No neurological abnormality was found on examination.

Case 3. J., a 21-year-old married spray-man, began working with dieldrin in April 1956 having had no previous contact with insecticides. He wore shorts and a vest. He had no shoes and no hat. No soap was supplied to the unit. He was aware of dieldrin on all parts of his body and claims to have bathed with soap at home before eating his evening meal. He had 9 attacks, characterized by convulsions and unconsciousness lasting about a minute. The fits occurred as follows: August or September 1957, 1; October, 1; December, 4; January 1958, 3. He continued to work with dieldrin during this entire period and it was not until March 1958 that he was transferred to DDT spraying. At least some of the 9 attacks were treated in a polyclinic with a diagnosis of malaria. There was no history of previous fits in the man or in his family. On 3 July 1958 he stated that he felt well. The neurological examination was normal.

Case 6. A., a 20-year-old spray-man, began working with dieldrin in March 1957. His clothing and bathing habits were similar to those of other spray-men. According to a diagram prepared by him and his supervisor, he had one fit in July, four in August, and three in September 1957. He worked with DDT during February, March and April 1958 and did office work during May and June. During June he had five fits, making a total of 13. Thus, these last convulsions were a little over four months after his last exposure to dieldrin.

Case 8. S., a 20-year-old spray-man, worked with DDT from July 1955 through March 1956 and with dieldrin from April 1956 until March 1958. He wore long trousers and a vest but no hat or shoes. Soap was not supplied at work. He washed without soap before eating at home and bathed with soap in the evening. His first attack was in December 1957. It was characterized by temporal headaches, momentary unconsciousness without falling and a sense of suffocation which did not interfere with work but which required deep breaths. There were several similar attacks. He denied that he or anyone in his family had had this trouble or frank convulsions. (The illness of this man appears to have been entirely subjective. It seems impossible to determine at this time whether he suffered from autosuggestion or from something analogous to *petit mal* epilepsy.) The neurological examination was normal.

Case 9. S., a 25-year-old married spray-man, began using DDT in September 1954 and changed to dieldrin in June 1956. He wore a sports shirt, shorts or long trousers, and a cap with a visor. He had no shoes. His history of bathing was similar to that of the others. He occasionally bathed in the fields where there was plenty of water available. He had a total of four fits, which occurred in June, July and October 1957 and February 1958. The first two fits were observed by his wife. The fits were accompanied by headache and nausea. After each attack, he was treated in a hospital out-patient department but the composition of the pills given is not known. In February 1958 the man was transferred to DDT spraying, after which he had no further attacks. There was no personal or family history of fits. The neurological examination was normal.

Case 12. B., a 20-year-old spray-man, sprayed DDT from November 1955 through March 1956 and sprayed dieldrin from April 1956 until March 1958. He wore long-sleeved shirts and long trousers but no hat or shoes. He washed his hands without soap when he finished work and bathed with soap in the evening after eating. In February 1957 he had headache, nausea, and vomiting but no loss of consciousness. The headache had a bilateral temporal distribution. He had had headache before but not one of such long duration. He received treatment from several polyclinics but it was not effective in relieving the headache or other symptoms. The neurological examination was normal.

Case 15. K., a 20-year-old spray-man, worked with dieldrin for 10 months before becoming ill in October 1957. His clothing and bathing habits were similar to those of his fellows. His supervisor saw him have a fit, which lasted five minutes and came without apparent warning. While the patient was cycling to the hospital one hour later, he fell and had another fit. It was followed by headache, weakness and nausea. He rested for two months after leaving the hospital and then began work with DDT. There was no personal or family history of epilepsy.

Case 16. S. was dismissed from work when he had his first fit in August 1957. He had worked with dieldrin since March 1957. He had two more fits during the week following his dismissal. He was then treated by a doctor. Later he returned to his home village. He is now well. There was no history of epilepsy in the spray-man or his family.

Case 17. K. began spraying dieldrin in April 1956 and continued until April 1958, when he transferred to DDT spraying. He wore shorts and a vest. He had a cap with a visor but no shoes. No soap was available at work. After going home on his bicycle he washed his hands with soap before eating. Later he bathed with soap. He had three attacks, all of which were on a single day in November 1957. He fell from his bicycle on the way to work and hurt his leg. He was momentarily unconscious and woke with headache and nausea but no vomiting. While at work he had another attack which was seen by the owner of a house. He was sent home by his supervisor. Having reached home, he fell again while on his way to the river to defaecate. It was reported that he had no convulsion during his brief periods of unconsciousness. Neurological examination, done on 3 July 1958, was normal.

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Six of the above cases (Nos. 1, 3, 8, 9, 12, 17) were from the Kendal area, where 13 men were said to be sick out of 15 squads (15 supervisors, 15 mixers, 75 spray-men).

It is noteworthy that the Regency Supervisor stated to me that he had never seen anyone have a fit except the men listed as cases 15 and 16. He was in charge of 50 spray-men (10 squads), among whom three (including cases 15 and 16) had been poisoned.

During the period when cases of poisoning were reported from Central Java only, there were many speculations to explain the supposed distribution. It was alleged that the spray-men in Central Java were more careless, that supervision was poor in some areas, that there had been a drought so that it was impossible for the men to bathe or obtain adequate food during the period when illnesses occurred and, finally, that illnesses had occurred in different areas in direct proportion to the amount of dieldrin used. Although it was not possible to investigate each of these explanations fully, I encountered no evidence that any of them was valid. There was little difference in the care which I saw used in spraying in West and Central Java. There is no significant relationship between the reported occurrence of poisoning and the amount of dieldrin used (see the tabulation on page 895).

The true difference lay in reporting rather than in other factors. The physicians who had observed cases of dieldrin poisoning in West Java and in East Java had not yet reported their findings to the leaders in Djakarta.

The Residency Physician for West Java stated that he had treated mixers and spray-men from several squads who had suffered sudden falls and convulsions. The fits lasted 5 to 10 minutes. They involved foaming at the mouth and rolling up of the eyes. Fits were usually followed by giddiness, headache, and vomiting. The patients were treated by giving them, while they were still unconscious, the juice of a sour fruit which is commonly available in Indonesian kitchens. The affected workers were given a two-week rest period and then returned to dieldrin spraying. At least one man had a second fit about a month after returning to work. The initial attacks followed 6-8 months of exposure to dieldrin, except in one instance in which a connexion between the man's smoking habits and the early onset of sickness was suspected. The first recognized case occurred in December 1956, 6 months after spraying with dieldrin was begun in June. Similar illness was unknown in the area before dieldrin was used.

The Residency Physician estimated that 14 of the 35 spray-men in his immediate area had been made sick by dieldrin. Reports of similar illness had come to him from other parts of the Residency and he considered poisoning by dieldrin to be a great problem in West Java, even though no human deaths had occurred.

In East Java, 15 cases of dieldrin poisoning were observed by the Deputy Inspector of Health, Dr M. Imanoedin (personal communication, 1958) among 833 labourers of all categories up to September 1958. One case appeared after 6 months; two after 7 months; two after 8 months; one after 9 months; three after 10 months; two after 11 months; one after 12 months; two after 14 months; and one after 15 months. Illness was characterized by headache, blurred vision, insomnia, general malaise, sudden falls, and, in all instances, convulsions with loss of consciousness. Examination of blood, urine, and faeces in all the patients and complete neurological examination of three of them failed to reveal any abnormality.

As with cases of poisoning in man, the poisoning of domestic animals was reported first in Central Java and it received more attention there than in other areas. However, it seems probable that there has been no major difference in the incidence of animal deaths in the three Provinces. Cats and mice are reported to have died following the use of dieldrin in West

Java; and in Central Java so many cats were killed that the price of cats more than doubled. The early reports about the death of chickens and other animals in Central Java were probably exaggerated. However, C. A. Ferullo (personal communication, 1957) has shown by experiment that a 5% mortality is to be expected in chickens from villages sprayed with dieldrin, and when the recommended precautions are not applied a much higher mortality is to be reckoned with. No evidence has been advanced to prove that dieldrin has been responsible for the deaths of horses, buffaloes or goats.

India, Bombay State

The use of dieldrin for malaria eradication in Bombay State was undertaken for experimental and other purposes unrelated to any resistance of the local vector. The use of dieldrin as an adulticide for filariasis control is also considered by some to be experimental.

All the important matters concerned with the occurrence of poisoning by dieldrin in Bombay State have been published by Patel & Rao (1958) and will not be repeated here. However, a few points are discussed. Incidentally, 5 cases of poisoning have been reported from another part of India (Rahman et al., 1958).

On direct observation, it is evident that the spray-men in Bombay State have a relatively greater part of their bodies exposed than workers in most other parts of the world. This is largely necessary because of the hot, wet climate. However, this exposure of a large skin area may well have been an important factor in permitting the occurrence of poisoning in spite of relatively short cycles of spraying (32, 71 and 78 days respectively) and relatively long intervals between cycles (98, 63 and 31 days respectively). Spraying is timed according to the monsoons. No relationship between personal bathing habits and susceptibility to poisoning has been noted.

The use of dieldrin for malaria eradication was stopped from late November to 16 June 1958. During this period protective clothing was designed and made from the plastic liners of the drums in which dieldrin is shipped. The clothing consisted of caps with visors and coats of approximately knee length with three-quarter-length sleeves. The legs and feet of the workers were uncovered and the caps offered, at best, only partial protection to the face and neck. Protection of the lower part of the face was given by a surgical mask. The spray-men were equipped with "bent nozzles", i.e., spray wands bent at about a 45° angle about 7-10 cm below the attachment of the nozzle.

Inspection of 25 of these workers at Padgha near Thana at the end of a day revealed that in many instances their feet and legs were white with spray residue. Some of them had grossly visible deposits of wettable powder on their arms and hands beneath their gloves. Although the men had been shifted to DDT some time earlier, experience indicates that in spite of the best efforts of supervision, their use of dieldrin may not have differed greatly from their use of DDT.

The same observations largely held true for spraying which was observed in another village (Manelau, at some distance from Poona). The spray-men were disciplined and efficient, but the fact that they were not completely covered brought them in direct contact with more spray mist than necessary. Furthermore, houses were not cleared before spraying. Cooking vessels and trays were sprayed freely. It was claimed that the food vessels were all washed. DDT was being used but with dieldrin a single contamination of food would be dangerous. During the spraying process, children wandered in and out and some of them got visible spray on their skin and hair. Some of the compression sprayers were equipped with constant pressure devices adjusted to 10 p.s.i. (0.7 kg per cm^2). They were considered entirely satisfactory. Most of the sprayers available were not equipped with such devices and were operated at pressures of 20-40 p.s.i. (1.4-2.8 kg per cm^2).

Since the published report by Patel & Rao (1958), there have been further developments which will be reported by those investigators. Briefly, a detailed laboratory and neurological examination has been made of four of the original ten patients from the malaria control team, and new cases have occurred following a temporary return of the workers to dieldrin spraying.

Neurological examination of four men, 3-4 months after their last exposure to dieldrin, showed completely normal findings. Only one of the men showed a dysrhythmic electro-encephalogram, while another had an epileptic fit that was observed by a nurse in the hospital. Various laboratory tests including liver function tests and examination of the cerebrospinal fluid were normal in the four men, but three of them showed an eosinophilia ranging from 8% to 34%, which is unusually high for the area. One of the patients gave a history of unconsciousness without jerking.

The occurrence of five new presumptive cases (one involving a fit) following the reinstatement of dieldrin on 16 June led to a return to DDT on 28 June.

Patel & Rao pointed out that dieldrin had been used in Bombay State for periods of 5 months or less in 1955 and 1956 without any reports of toxicity. They felt, however, that the possibility that poisoning did occur could not be completely ruled out.

These authors have published their hypothesis that dieldrin poisoning involves an allergic phenomenon. The matter is certainly worthy of consideration, but several facts need to be kept in mind in reaching a final decision. Of the 20 cases reported, five occurred in the first cycle during which the worker was exposed (14, 16, 26, 36 and 60 days respectively). Since the longest spray cycle reported was only 78 days and since it has frequently taken several months of exposure in other programmes to produce dieldrin poisoning, it may require no special explanation that the other cases did not occur in the first spraying cycle during which the worker was exposed. Furthermore, the cases which occurred in a worker's second or third spraying cycle did not appear on first re-exposure, as one would expect in case of hypersensitivity, but only after several days or even weeks.

In case No. 4 reported by the same investigators, a fit followed exposure to BHC some 8 months after the last exposure to dieldrin. This relationship may have significance. However, there have been relapses in some patients who have been poisoned by dieldrin and who have had no further exposure to dieldrin or other insecticides. Thus the possibility must be considered that the fit in case No. 4 was in the nature of a relapse in a previously unrecognized case.

Iran

Dieldrin has been used for indoor spraying only in the southern portion of the country, where the malaria vector has developed resistance to DDT. The compound has been used for two cycles of 60 days or less, separated by an interval of 5 months. The most recent spray cycle was completed in April 1958. About 1740 spray-men and 174 mixers were exposed but only for these short periods. Their bodies were fully covered but their hands and faces were generally bare. They were given free soap, and they washed before eating and after work.

Spraying of DDT was in progress in the Teheran area in July 1958. The workers were well protected except that their faces, hands and ankles were bare. They were careless in handling the wettable powder and suspension with the result that their hands were frequently soiled either in mixing the suspension or in transferring it to the sprayers. During the spraying some vessels for food were contaminated and the mangers from which animals ate were sprayed thoroughly.

It was felt that the plastic veil would be acceptable to spray-men in Iran partly because of its similarity to the head-dress worn by Arabs.

Discussion

Dieldrin is a toxic substance which is freely absorbed by the skin. Dry dieldrin is absorbed about as easily as dieldrin put on the skin in solution. The insecticide is less than twice as poisonous by mouth as by skin application—an insignificant safety factor. Dieldrin differs very much from DDT in this respect. The oral toxicity of dieldrin is only 3-5 times greater than that of DDT. However, DDT is not readily absorbed by the skin even when in solution, and dry DDT is so little absorbed by the skin that toxicity is not evident.

The dermal toxicity of dieldrin has not been fully appreciated by some of those who have used the compound in antimalaria programmes. A relevant point which has been even more generally missed is that all of the unclothed skin of a spray-man (and, to a smaller extent, his protected skin also) is subject to constant contamination during the process of spraying. This " imperceptible " contamination is added to any visible soiling from spillage or other direct contact with dieldrin powder or suspension. Chemical measurements of " imperceptible " contamination during indoor spraying have emphasized the magnitude of the problem and also have shown the relative unimportance of respiratory exposure (Wolfe et al., 1959). These measurements are of special importance because it has commonly been assumed without any basis that the major source of dieldrin poisoning is respiratory exposure. This is an understandable mistake, because the great importance of gases, metal fumes, and silica dust in industrial medicine has led to great emphasis on respiratory exposure. It is also true that dieldrin is poisonous if it is inhaled. The crux of the matter is that only small particles can be inhaled, and nearly all the dieldrin in water-wettable powder is impregnated on particles too large to be inhaled. The spraying process does not grind the powder any finer. On the contrary, wetting the powder makes it more dense and spray droplets may be appreciably larger than the powder particles which they contain. It follows that measurement of the concentration of dieldrin in the air during spraying may be entirely misleading unless the particle size and the fact of dermal toxicity are taken into account.

If one assumes that man is as susceptible to dieldrin poisoning as are experimental animals, then the " imperceptible " mist of dieldrin suspension which falls on the unprotected face and lower arms of spray-men is sufficient to account for the incidence of poisoning which has been observed. This is not astonishing in view of the fact that during the working day the spray mist, which is imperceptible as it falls, often accumulates on the exposed skin to form an easily visible residue.

Another technical point is that any given dosage of dieldrin is absorbed more readily by a large area of skin than by a small area. This means that any increase in the area of skin exposed not only increases the dosage that will be received but facilitates absorption.

Poisoning of spray-men and mixers using dieldrin has been recognized in Venezuela, Ecuador, Nigeria, Java, and India. Informal reports indicate that it has occurred also in other places. Poisoning by dieldrin has not occurred in the Nandi or Taveta-Pare projects and has not been reported from Iran, Liberia, or the Philippines, where this insecticide is also in use.

The failure of poisoning to occur or to be reported in some places where dieldrin has been used is encouraging but—for very different reasons in different instances—must be viewed with scientific conservatism until further information is available.

Many of the cases which have been recognized as dieldrin poisoning have involved serious illness. The proportion of recognized cases showing one or more epileptic fits has varied from 47% to 100% in different countries. The occurrence of fits in a very high percentage of cases almost certainly means that some less severe cases were missed. In fact, there is no assurance that the complete spectrum of dieldrin poisoning is known at this time. Among the cases of dieldrin poisoning, there are at least six men who had one or more fits 15 to 120 days following the last exposure to dieldrin. It is probably too early to assume that some of these men will not have another fit. Furthermore, because it has not been possible to follow all cases of recognized poisoning even for a few months after dieldrin exposure had ceased, it is probable that the recurrence of symptoms was missed in some cases. Recurrent convulsions have been observed in experimental animals and occasionally persist until the animal dies at an old age. In addition to sudden falls, unconsciousness, and convulsions, another disturbing sign in some cases of dieldrin poisoning in man and animals is mental disorder. In man, the trouble has taken forms ranging from loss of memory, insomnia and nightmares to mania. It is reported that two men have died in convulsions following exposure to dieldrin. The cases have not yet been properly studied. It is therefore not possible to state whether either of them showed a syndrome clinically similar to that of poisoning observed in animals, which was characterized by complete loss of appetite and consequent severe weight loss and which was uniformly fatal in the absence of medication.

It is true that, after having a fit, some spray-men have continued their occupational exposure to dieldrin without disastrous effects. However, the recurrence of symptoms in men and animals after exposure has stopped raises the possibility that dieldrin produces a morphological and/or biochemical lesion which persists for some time and may give rise to recurrent illness. It may be assumed that, during exposure, dieldrin circulates in the blood and becomes stored in the fat. Unfortunately, practically nothing is known of the dynamics of dieldrin storage. It is therefore impossible to state the relationship between storage and the observed recurrence of characteristic illness following long intervals without re-exposure.

When poisoning by dieldrin has first been observed in a country, it has been a common practice to suppose that the victims were susceptible because of some inherent weakness. Infestation with worms, poor nutrition, "epileptic tendency" and inbreeding are some factors which have been suggested. Such factors are worthy of scientific consideration, but so far not a shred of evidence has been presented that they have any significance whatever in determining the occurrence of poisoning under practical conditions.

A vast difference has been observed between the spraying practices by which dieldrin is applied in different countries. Some of the main points

COMPARISON OF DIELDRIN SPRAYING PROGRAMMES IN DIFFERENT COUNTRIES

Country or area	Concentration of dieldrin and formulation	Area of spray-man's body which is bare [a]	Gross exposure	Duration of spraying		Interval between cycles (days)	Number of spray-men	Proportion of workers poisoned (%)	Proportion of cases involving fits (%)
				cycle (days)	total (days)				
Bombay State, India [b]	1.25 % suspension	Head, arms, lower legs	Mixed with hands	78	140	31	192	7	80
Bombay State, India [c]	2.50 % suspension	Head, arms, lower legs	Mixed with hands	32-71	160	63-98	105	10	100
Ecuador	2.50 %						92	9	50
Indonesia	1.25 % emulsion	Head, hands, lower legs	Frequent soiling of hands observed	365	852	0	145 [d] 35 [e] 833 [f]	11 [d] 40 [e] 2 [f]	88 [d] 100 [e] 100 [f]
Iran	1.25 % suspension	Face, hands	Disclaimed	45 [g]	90	150	670	0	—
Nandi, Kenya	1.7 % suspension	Hands, knee area (shorts and boots worn)	Minimal	90 [h]	270 [h]	275	90	0	—
Nigeria	0.68 % suspension and emulsion; 1.37 % suspension	Face	Frequent	77-98	341	77-99	40	10	100
Taveta-Pare	0.55 % suspension	None	Minimal	151-174	810	68-108	10	0	—
Venezuela	1.25 % suspension; 2.50 % emulsion	Face	Frequent soiling of hands in some groups	365	1460	0	285	18 [i]	47

[a] It is realized that parts of the body which are covered by some kind of clothing may really be exposed to dieldrin. This is especially important in the case of improperly used gloves.

[b] Antimalaria programme

[c] Antifiliariasis programme

[d] Central Java (values based on selected groups for lack of statistics for whole work force)

[e] West Java (values based on selected groups for lack of statistics for whole work force)

[f] East Java

[g] Maximum 60 days

[h] Maximum of 90 days for each worker

[i] For those working eight months or more, 26%; for one sub-group, 50%.

of difference are outlined in the accompanying table. Unfortunately, some of the values in the table are tentative. Furthermore, there can be no assurance that all of the important factors related to the safe use of dieldrin are now recognized. In spite of these handicaps, there is clear evidence of a direct relationship between dosage and the occurrence of poisoning. This is manifest in at least four ways:

1. The greater the concentration of dieldrin suspension used for spraying, the greater is the chance that poisoning will occur following a given period of exposure (see the table).

2. Under otherwise similar conditions, the greater the area of bare skin exposed during spraying, the more likely is poisoning to occur (see the table).

3. The longer the exposure (at least up to 2 years) under conditions which lead to some poisoning, the greater will be the total incidence of poisoning (Hayes, 1957). It seems certain that intervals between cycles of exposure allow some excretion of stored dieldrin, but the rate of such excretion is unknown. Rest periods may also permit some degree of recovery from other possible effects of dieldrin. The fact remains that the practical importance of rest periods in preventing dieldrin poisoning is not known.

4. Immediate washing of the contaminated skin is partially protective; delayed bathing offers distinctly less protection (Hayes et al., 1951). Although one may assume on logical grounds that the absorption of dieldrin continues as long as the compound is in contact with the skin, there is no objective evidence that washing which is delayed for several hours or more after exposure has any protective effect whatever.

Because of differences in one or more of the dosage factors just listed, the exposure of spray-men in those programmes under study which have not given rise to reported poisoning is recognizably less than that in those programmes in which poisoning has occurred. There is no evidence which would lead one to predict the occurrence of poisoning in those programmes in which it has not already occurred, provided the conditions of exposure remain unchanged. On the other hand, there are several reasons why it is impossible to guarantee that poisoning will not appear later even under the same conditions.

It is a general principle of toxicology that even the most poisonous compound may be used if adequate safeguards are provided. It does not follow that adequate safeguards can be provided under all conditions. One must face the logical possibility that some programmes, for reasons of climate, or finance, or the availability of competent supervisors, cannot provide the training, supervision and protective equipment necessary to reduce the incidence of poisoning among spray-men to a minimum. It is worth mentioning, however, that the preconceived ideas of programme leaders have sometimes been important in causing the rejection of good equipment, which had proved acceptable under similar conditions in other places.

Recommendations

It is clear from the record of use that dieldrin is a dangerous compound. It should not be used for indoor spraying unless resistance of the vector to safer insecticides, or some other good reason, makes dieldrin really necessary for disease control. If those responsible for vector-control programmes consider it necessary to use dieldrin, then they have a responsibility to recognize the calculated risk. The present survey justifies the belief that the risk may be minimized, but not necessarily eliminated, by certain protective measures. These measures are consistent in principle with those previously recommended by the World Health Organization (1956) but are arranged here in approximate order of increasing expense. It appears that some programmes have not used all the facilities at their disposal to ensure the safety of their spray-men.

Suggested protective measures are as follows:

(1) Each worker should be told when he is hired that he is to work with a dangerous compound which has produced serious sickness in men like himself. Dieldrin poisoning and the protective measures which will be required should be described.

(2) Rigid supervision should be maintained to ensure the practice of:

(*a*) washing the hands at frequent intervals even though no contamination is visible;

(*b*) washing the hands and face with soap and water before meals and before smoking;

(*c*) washing with soap and water after each recognized contamination of the skin;

(*d*) bathing the entire body with soap and water at the earliest practical moment after work is finished for the day. It is sometimes stated that there is inadequate water for washing; to the toxicologist, there is always enough water for washing if there is enough for spraying. The cost of hauling the water simply must be counted in the cost of spraying.

(3) Free soap should be available for the washing recommended under items 2 and 4. It was found on one programme that a ration of 250 g of soap per man per week was adequate. In another programme, new soap was issued to each man when he exhausted his previous supply.

(4) Work clothing should be washed daily with soap and water. If this daily laundry is not done by an outside contractor, then it becomes a responsibility of each spray-man and must, of course, be done during working hours and under the supervision of a foreman, like any other duty for which the spray-man is employed.

(5) Arrangements should be made for each spray-man (including pumpers and mixers) to have at least two sets of work clothes with a high neck-line, long sleeves and long trousers. The work clothes are not to be worn after working hours. The clothing must not be worn with the sleeves or trousers rolled or with the collar open.

(6) Arrangements should be made to ensure that spray-men wear shoes and socks.

(7) Each worker should have a broad-brimmed, water-repellent hat. Hats of local manufacture that satisfy these requirements are acceptable and frequently very much cheaper than imported hats.

(8) A veil of plastic netting such as that described by Wolfe et al. (1959) should be attached to the hat in order to protect the face, neck and shoulders of spray-men in those projects where the climate or some other factor prevents the wearing of a plastic face shield. (A visor such as those on caps worn by tennis players does not give adequate protection.)

(9) Rubber gloves are desirable only if they are used properly. Gloves which are contaminated on the inside are a source of added danger rather than protection. The hands should be washed before gloves are put on and gloves should be washed *before* they are removed.

(10) A short (arm-length) cape of plastic sheeting or cotton cloth offers some added protection to the clothed areas beneath it. A cape cannot be considered a substitute for any of the equipment listed under items 1 to 9.

It would be unrealistic to assume that adequate protection can be achieved by substituting bathing for clothing. As already mentioned, the bare head and lower arms provide enough surface for receiving a poisonous dosage of dieldrin in the course of several months of spraying. A larger area of bare skin increases the hazard. If dieldrin reaches the skin, some of it will be absorbed before it can be washed off. Therefore washing and bathing must be an important second line of defence to remove as completely as possible any insecticide which has penetrated the clothing or other protective equipment.

This emphasis on dermal exposure and absorption is not to suggest that dieldrin is not poisonous by other routes. Respiratory toxicity is unlikely to occur only because of the unlikelihood of exposure to a sufficient density of dieldrin-bearing particles 1-5 microns in diameter. The possibility of poisoning from eating dieldrin is much greater than the possibility of poisoning from inhaling it. It takes only about half as much dieldrin to produce sickness if it is eaten as it does if it is put on the skin. On the other hand, the contamination of the exposed skin of spray-men while they work is inevitable and continuous. The need for protection is not obvious to the ordinary labourer, and even the most practical protective equipment produces some discomfort. By contrast, the need to avoid eating dieldrin is obvious and the means for doing this are simple and involve no real discomfort, although it may take some self-restraint to delay eating, drinking, or smoking until the hands have been washed and until a suitable, uncontaminated place has been found, and it takes a little planning to transport lunches, drinking-water, and tobacco without contaminating them. The possibility cannot be excluded that some cases of occupational poisoning have involved ingestion of dieldrin. However, in studying different programmes it has not been possible to associate poisoning definitely with eating, drinking, or smoking habits. Finally, as already mentioned, the measured contamination of the skin is adequate to explain the observed clinical result.

In the present limited state of knowledge, it seems only reasonable that a person who has shown dieldrin poisoning once should have no further contact with the compound. He should, however, be followed medically for a minimum of two years to determine whether he will show any sequelae. A much longer follow-up is, of course, desirable. It should be noted that all the human cases which have been well described were of the type which, in animals, show spontaneous recovery (albeit with occasional recurrences) without medication. This does not mean that patients should not receive medication. On the contrary, there is evidence that the comfort (if not the survival) of patients can be improved by the same kind of medication which can mean the difference between life and death in more severe poisoning by dieldrin in animals. The treatment includes barbiturates and a proper diet. Phenobarbital is the drug of choice because of its long action; patients should be given as much of this drug as they will tolerate without sleeping more than the usual amount. The diet should be a good one given in sufficient amount to maintain or even increase the body weight.

In addition to protective measures which apply to the individual worker, other factors need to be taken into account:

(1) Sprayers must be kept in repair so that they do not leak.

(2) Measuring cups, mixing buckets, funnels and other devices must be provided with handles which will permit the workers to use them without ever touching dieldrin powder or suspension.

(3) Sprayers should be used at the lowest pressure consistent with vector control. Wolfe et al. (1959) have shown that the contamination of the worker is much greater at a pressure of 50 p.s.i. (3.5 kg per cm^2) than at 20 p.s.i. (1.4 kg per cm^2).

(4) At least one professional man should be appointed safety officer, with no other responsibility and with full power to enforce regulations. The appointment of such an officer will greatly support the authority of foremen who accompany spray-men during the entire working day. It is understood that discipline cannot be adequate unless each spray-man is subject to summary discharge if he violates safety rules and unless each foreman is also subject to discharge for failure to carry out his duty in regard to safety.

(5) Spray-men and mixers should not be permitted to work more than eight hours per day or 40 hours per week.

(6) It must be frankly admitted that (in terms of wall area covered and excluding the cost of insecticide) it is more expensive to apply dieldrin safely than to apply an insecticide like DDT safely. Increasing the pay of spray-men would improve their morale and help to ensure their co-operation in safety regulations.

There is an urgent need for further study of dieldrin to clear up some of the uncertainties which now exist about this very useful insecticide. It is not known how long is required for a single species to reach equilibrium of dieldrin storage. This information is needed to explain the relationship between dieldrin storage and toxicity. It might throw light on the recurrence of symptoms after cessation of dosage and also clarify the hypothesis about allergy to dieldrin. Species may differ widely in the rate at which they reach a steady state in the storage of a compound. Thus, the information on storage is needed eventually in relation to man himself.

Although a method exists for the measurement of dieldrin in fat, methods are not available for measuring dieldrin or any derivative in the blood or the excreta. Such methods should be devised, if possible. The appropriate measurement of dieldrin-derived material in the blood or urine of spray-men should give information on:

(*a*) the relative amount of dieldrin absorbed under different conditions of spraying;

(*b*) the time necessary to reach an equilibrium of excretion; and thus

(*c*) the relation between toxicity, dosage, and time.

Especially in view of the present lack of a method for measuring dieldrin in blood or excreta, it would be highly desirable if measurements similar to those reported by Fletcher et al. (1959) could be made in a number of dieldrin-spraying programmes. It is true that Wolfe et al. (1959) have measured the effectiveness of protective clothing and devices employed by a single skilled spray-man. However, similar information should now be obtained under a variety of field conditions involving differences of climate, labour force, etc. It is entirely possible that study of such measurements would make it possible to state with greatly increased confidence that certain spray practices are safe.

It has been claimed that by clinical histories and neurological examinations done at intervals, it is possible " to detect incipient intoxication by dieldrin before serious manifestations occur " (Winthrop & Felice, 1957). The possibility is a very attractive one, worthy of the most careful scientific work to confirm or refute it. Unfortunately, the conditions of such a study are complex. It is necessary that the investigators be versed in statistical tests of probability as well as in clinical medicine. It is also necessary that a programme be selected for study which has given rise to a significant incidence of poisoning in the past and in which the initial conditions of exposure of the workers can be continued without change during at least two years of investigation. Of course, if cases of clinical poisoning continued to appear under these conditions, the limited usefulness of the test would be revealed in a shorter time.

RÉSUMÉ

La dieldrine est de plus en plus utilisée dans la lutte contre le paludisme pour plusieurs raisons, dont une est la résistance sporadique au DDT de quelques vecteurs. L'intoxication par la dieldrine des hommes qui vaporisent cet insecticide a été observée en Equateur, en Inde, au Nigeria, au Venezuela et plus récemment en Indonésie. D'autres cas ont été signalés dans plusieurs autres pays. L'auteur décrit quelques cas d'intoxication en Indonésie.

Les risques liés à l'utilisation de la dieldrine sont proportionnels au degré d'exposition des travailleurs, lui-même déterminé par la concentration, la surface cutanée dénudée, le temps de contact et le manque d'hygiène: tels sont les enseignements des programmes de lutte antipaludéenne au Kenya, au Tanganyika, en Indonésie, dans l'Etat de Bombay, en Iran et ceux de la littérature s'y rapportant. Si les risques d'inhalation de dieldrine sont insignifiants dans les conditions habituelles de travail, si l'ingestion en est dangereuse, et tout travailleur comprend bien qu'il faut l'éviter, par contre la nécessité d'éviter l'exposition cutanée n'a pas été assez bien comprise, même par les dirigeants des programmes de lutte. Les moyens de mesurer l'exposition des travailleurs à la dieldrine et les recherches sur les différentes voies possibles d'intoxication montrent que la contamination par la peau constitue en pratique le danger le plus grand. Le produit est à peine plus toxique par la bouche que par application cutanée. La peau absorbe avec autant de facilité la dieldrine en poudre que la dieldrine en solution. La toxicité orale de la dieldrine est seulement trois à cinq fois plus forte que celle du DDT. Cependant, le DDT n'est pas facilement absorbé par la peau, même en solution, et le DDT en poudre est si peu absorbé de cette façon que sa toxicité par cette voie n'est pas patente.

Si l'on suppose que l'homme est aussi sensible à la dieldrine que les animaux de laboratoire, le nuage «imperceptible» de dieldrine qui adhère au visage non protégé et sur les avant-bras des hommes qui vaporisent est suffisant pour expliquer la fréquence des intoxications observées. Ceci n'est pas étonnant quand on pense qu'au cours d'une journée de travail le nuage de vaporisation, s'il est imperceptible quand il retombe, finit par couvrir la peau d'une pellicule bien visible.

La dieldrine ne doit donc pas être utilisée à la légère; si son utilisation est indispensable, des mesures individuelles et collectives de protection peuvent diminuer les risques, sans forcément les éliminer. Il faut donc dire à chaque travailleur les dangers de la dieldrine et la nécessité d'une collaboration de sa part dans les mesures de protection. Il faut surveiller le lavage *a*) des mains à intervalles fréquents, *b*) des mains et du visage avant les repas ou avant de fumer, *c*) de toute surface cutanée visiblement atteinte, *d*) de tout le

corps après le travail. Il faut exiger ou procurer *a*) du savon à discrétion, *b*) une lessive quotidienne des vêtements de travail, *c*) au moins deux jeux de vêtements (uniquement destinés au travail) à col montant assez haut, à manches longues et à pantalon long, *d*) un chapeau à larges bords, *e*) un voile en filet de plastique comme ceux décrits par Wolfe et al. pour les programmes de lutte où un masque en plastique ne peut être utilisé du fait du climat ou pour d'autres raisons. Des gants en caoutchouc ne sont souhaitables que s'ils sont utilisés à bon escient. Une courte pèlerine peut augmenter la protection des vêtements qu'elle recouvre. Enfin, à ces mesures, il faut ajouter l'entretien des pulvérisateurs et la nécessité de munir de poignées les récipients de mesure, les seaux et autres ustensiles que les travailleurs doivent pouvoir manipuler sans toucher à la dieldrine. La pression des pulvérisateurs sera la plus basse possible, compatible avec la lutte contre les vecteurs. On devrait toujours désigner un responsable de la sécurité, le travail ne devrait durer que huit heures par jour et 40 heures par semaine. Il faut d'autre part tenir compte franchement des dépenses supplémentaires qu'entraîne l'utilisation en toute sécurité de la dieldrine. Il est urgent d'entreprendre des études complémentaires sur la dieldrine, notamment pour éclaircir certaines incertitudes que comporte encore cet insecticide, pourtant des plus utiles. Il faudrait en particulier *a*) étudier la dynamique d'accumulation de la dieldrine dans n'importe quelle espèce et par la suite chez l'homme, *b*) mettre au point une méthode quantitative de mesure de la dieldrine, ou d'un dérivé, dans le sang et les urines de l'homme, *c*) trouver les moyens supplémentaires de mesurer le degré d'exposition des hommes qui vaporisent, dans les différentes conditions de travail et *d*) faire une étude critique de la valeur de certains procédés de diagnostic des intoxications légères ou imminentes.

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CONCENTRATION OF O,P'-DDT IN RAT BRAIN AT VARIOUS INTERVALS AFTER DOSING

BY

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(Received for publication 2-8-1965).

DALE *et al.* (1) showed that the severity of signs of poisoning in rats after a single acute oral dose of *p,p'*-DDT [1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane] was directly proportional to the concentration of *p,p'*-DDT in their brains.

Since DOMENJOZ (2) and VON OETTINGEN and SHARPLESS (3) have shown that the acute toxicity of *o,p'*-DDT [1,1,1-trichloro-2,2-bis-(*o,p'*-chlorophenyl) ethane] to mice is 5 to 8 times less than that of the *p,p'*-isomer, two hypotheses may be considered: (a) the brain may be less permeable to *o,p'*-DDT and (b) the *o,p'*-isomer may be less toxic because of a more rapid conversion to one or more metabolites including *o,p'*-DDE [1,1-dichloro-2,2-bis-(*o,p'*-chlorophenyl) ethylene]. These hypotheses were evaluated in rats.

MATERIALS AND METHODS

Thirty-three adult Sherman strain rats weighing 250-300 g were used. The rats had been reared on Purina laboratory chow with no DDT added; they were fasted overnight before dosing with *o,p'*-DDT. Twenty-four of the rats were given a single dose of 3 % *o,p'*-DDT in peanut oil by stomach tube at a rate of 150 mg/kg of body weight. Three of the rats were dosed in the same manner but at a rate of 3000 mg/kg of body weight. Six rats were used as controls.

All rats were observed continuously for symptoms of poisoning during the first 9 hours after dosing. The rats that were dosed at the rate of 150 mg/kg of body weight were sacrificed in groups of six at 2, 5, 9, and 26 hours after dosing. The 3 rats dosed at the rate of 3000 mg/kg of body weight were sacrificed 9 hours after dosing. Control rats were also sacrificed along with each group of dosed animals.

Immediately after each rat was sacrificed, the brain was removed, ground with anhydrous sodium sulfate, and extracted with *n*-hexane. The *n*-hexane extracts were then passed through a modified DAVIDOW (4) column in which hexane had been substituted for carbon tetrachloride. About 250 ml of the eluate were collected and then evaporated to a convenient volume for injection into a microcoulometric gas chromatograph of the type described by COULSON *et al.* (5). A 10-foot aluminum tube, 3/16 inch in diameter, packed with 5 % QF-1 on 60/80 mesh chromosorb G. AW which had been treated with DMSC was used as the partition column. The temperature of the column was maintained at 162° C; the inlet and outlet blocks were maintained at 200° C. Nitrogen was used as a carrier gas, and the flow rate was adjusted to 100 ml/min at a pressure of 70 psi. The effluent gases from the column were oxidatively decomposed at 900° C and titrated coulometrically. A Sargent recorder with a full scale range of 12.5 mv and a chart speed of 0.5 in/min was used for recording the chromatograms. Under these conditions, the retention times of *o,p'*-DDE and *o,p'*-DDT were 15.3 and 26.3 min, respectively.

RESULTS AND CONCLUSIONS

The concentration of *o,p'*-DDT found in the brain of rats is shown in Table I. Rats given *o,p'*-DDT at the rate of 150 mg/kg of body weight

TABLE I

The concentration of o,p'-DDT in the brain of male rats at various time intervals after administration of a single oral dose of the compound.

Dose (mg/kg of body wt.)	Time after dose (hours)	Number of rats	Clinical signs of poisoning	Statistical value	*o,p'*-DDT (ppm) Whole brain	*o,p'*-DDT (ppm) Brain lipids
None	–	6	—		< 0.3	< 4.0
150	2	6	None	Range Mean ± S.E.	3—7 4 ± 0.6	42—98 60 ± 8
150	5	5	None	Range Mean ± S.E.	25—40 33 ± 2.5	333—526 434 ± 33
150	9	5	None	Range Mean ± S.E.	17—55 33 ± 6.8	222—727 438 ± 90
150	26	6	None	Range Mean ± S.E.	2—6 3 ± 0.6	21—76 46 ± 8
3000	9	3	Increased excitability to moderate tremor	Range Mean ± S.E.	121—214 153 ± 30	1610—2843 2036 ± 404

showed a significant increase in the concentration of this isomer in the brain — the highest level being observed at 5 and 9 hours after dosing. Twenty-six hours after dosing, the concentration in the brain had returned to a level equal to or lower than that found 2 hours after dosing. The concentrations of *o,p'*-DDT in the brain at various intervals after dosage was in good agreement with that reported by Dale *et al.* (1) for *p,p'*-DDT given at the same dosage. However, at similar brain concentrations the *p,p'*-isomer produced toxic symptoms, while the *o,p'*-DDT had no noticeable effect. The concentration of *o,p'*-DDT in the lipids of the brain required to produce excitability was 1610 ppm, while as much as 2843 ppm was required to produce moderate tremors. These concentrations were 5 to 9 times those of *p,p'*-DDT required to produce similar symptoms. Similar to the findings of Dale *et al.* (1) with *p,p'*-DDT, the ethylene derivative of *o,p'*-DDT (*o,p'*-DDE) was not detected in any of the brains. If *o,p'*-DDE was present in the lipid of the brain, it was present in concentrations of < 4.0 ppm.

Therefore, under the conditions studied, the relatively low toxicity of *o,p'*-DDT as compared with *p,p'*-DDT is not due to a lesser permeability of the brain to the *o,p'*-isomer nor to an increased detoxification by conversion to *o,p'*-DDE. It appears that the *p,p'*-DDT is inherently more toxic than the *o,p'*-isomer.

Summary

At the same oral dosage of 150 mg/kg, *p,p'*-DDT produces severe illness in all rats and kills about half of them, but *o,p'*-DDT produces no illness. However, the concentration of *o,p'*-DDT in the brain at various intervals after dosing was about the same as that reported by Dale *et al.* (1) for *p,p'*-DDT given at the same dosage. At a dosage of 3000 mg/kg, *o,p'*-DDT produces mild to moderate illness and the concentration in the brain was 5 to 9 times the concentration of *p,p'*-DDT required to produce similar symptoms. The ethylene derivative of *o,p'*-DDT (*o,p'*-DDE) was not detected in any of the brains. Thus, *p,p'*-DDT appears to be inherently more toxic than the *o,p'*-isomer.

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Polychlorinated Biphenyls (Aroclor 1242): Effects of Uptake on *E. coli* Growth

PESTICIDE
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Environmental Protection Agency
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Introduction

Polychlorinated biphenyls (PCBs), similar to DDT in structure and physiological effects (7), are of concern to the scientific community because of their ubiquity (8), persistence (11) and possible toxicity. The alleged lack of biodegradability of this environmental pollutant has caused alarm and curtailment of this useful chemical. Work as early as 1963 by Cope (3) indicated that DDT, another chlorinated hydrocarbon, might be metabolized by several species of bacteria. Subsequently, other workers (2, 4, 9, 10) reported degradation of this insecticide by bacteria, fungi, and marine diatoms. Unpublished work performed in our laboratory (5) also indicated possible metabolism of certain PCB components by *Cylindrotheca closterium.* With the thesis established that DDT was indeed biodegradable and with evidence that PCBs may be biologically converted, the authors postulated that human flora may play an important part in the metabolism of DDT, PCBs, and other ecological contaminants.

Experiments were performed to study the effects of PCBs in vitro on a facultative organism, *E. coli,* common to human intestinal flora. This bacterium was also selected because it is the prime indicator of fecal contamination.

Materials and Methods

The study organism, *Escherichia coli,* was selected. because it is a common autochthonous, human intestinal aerobe, easily cultured, and is an accepted standard indicator of fecal contamination

Initial work screened a wide range (0–1000 ppm) of PCB concentrations, against *E. coli* by use of impregnated paper filter discs similar to those used to test antibiotic sensitivity. Impregnated discs with PCB concentrations of 0, .001, .01, .1, 10, 100, and 1000 ppm were placed on tripticase soy broth (TSB) agar plates which had been inoculated with *E. coli.* Experimentation indicated that 6 mm diameter discs cut from number 2 Whatman filter paper would absorb .02 ml of the acetone solvent. The discs were dried prior to use.

For the first experimental series (I), 20 one-liter Erlenmeyer flasks, each with 500 ml. TSB, were inoculated with *E. coli.* Four flasks served as controls, 4 for each of 2 levels of PCB treatments, and 4 as acetone controls since acetone was the suspending vehicle for the toxicants. Additionally, 4 flasks were used as uridine controls. One flask, containing only media, was used as a growth check. Each flask was vigorously agitated every 2 hours during the first and last 8 hours of culture time.

At the conclusion of a 24-hour incubation period, flask contents were centrifuged, the resulting pellet lyophilized, weighed and assayed for PCBs, nucleic acids and tritiated uridine (^3H_U) uptake.

A second experiment (Series II), identical in treatments except for uridine, was conducted to confirm stimulation patterns observed in Series I. Series II measured lyophilized harvest weights only.

A third trial (Series III), identical in design to

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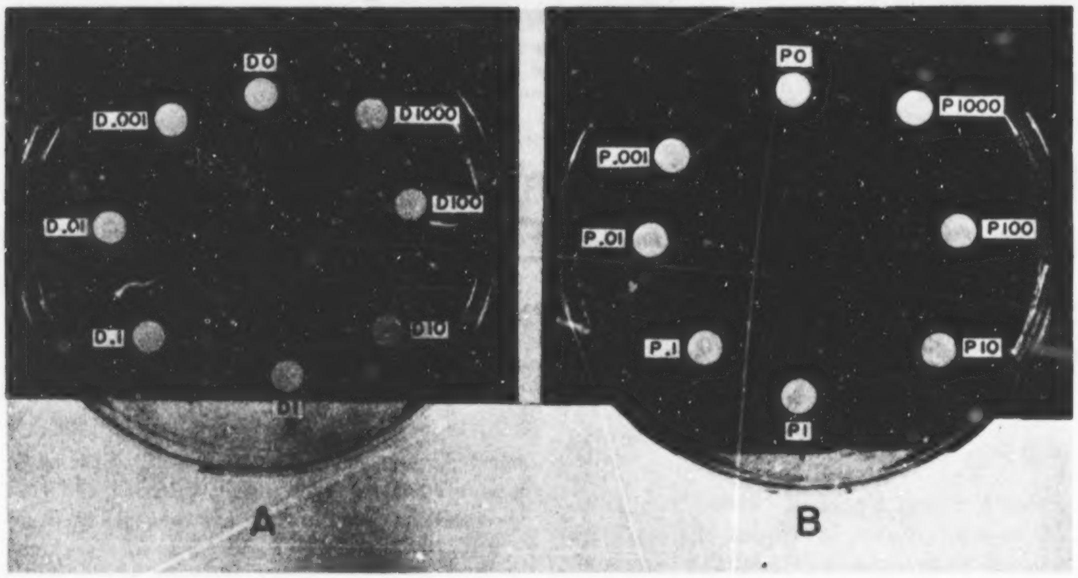

Figure 1. Sensitivity screening of *E. coli* on TSB to 0, .001, .01, .1, 1, 10, 100, and 1000 ppm of A, DDT; B, PCB.

Series I, assayed tritiated uridine uptake after 5 hours and 8 hours incubation time to determine if assimulation rates were affected by PCBs during the logarithmic growth phase. Aroclor 1242 (PCB—42% chlorine) was used as the source of active ingredients in these experiments.

Samples of bacteria were extracted with nanograde hexane and a 5 μl aliquot was injected into the gas chromatograph for analysis.

Two-column systems were used: a 6 foot × ¼ inch O.D. glass column packed with 3% SE-30 on 60–80 ABS chromosorb W and 2% QF-1 on 60–80 ABS chromosorb W. The operating parameters were: (1) inlet temperature 235°C; (2) column temperature 200°C isothermal; (3) detector temperature 240°C; (4) flow-rate 70 cc per/min. N_2.

Table 1. Lyophilized Weights and Toxicant Uptake by *Escherichia coli* Cultures Exposed to PCB (Bacterial Series I).

Treatment	Mean[1] harvest weights (mgm)	Mean[1] residue (μg/g)
PCB .1 μg/ml	86.0	13.6
PCB .01 μg/ml	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD$_{95}$[2]	13.6	10.8

[1] Mean of four replicates.

[2] LSD$_{95}$ = Least significant at 95% probability level calculated from one way analysis of variance.

Qualification was accomplished by measurement of the absolute and relative retention times of 5 major peaks of the PCBs. Quantitation was accomplished by determination of the area under the curve of the five major peaks. Utilizing this technique, recoveries of 92.4% of the PCBs were obtained.

RNA and DNA were measured using a modification of Agranoff's procedure (1). Radio assays utilized the Nuclear-Chicago Mark I Liquid Scintillation Spectrometer.

Results and Discussion

As may be seen from Fig. 1, concentrations up to 1000 ppm of PCBs impregnated into filter paper discs failed to inhibit *E. coli* growth on TSB agar.

As shown in Table 1 (Series I), the addition of .01 and .1 μg/ml PCBs to TSB markedly stimulated *E. coli* growth above control levels. This is consistent with other microorganism work (4, 6) showing that DDT and PCBs are concentrated up to 1000 times by marine diatoms. The data also suggest that the concentration factor may be inversely related to dosage.

Table 2 presents data from a duplicate experiment (Series II), wherein only harvest weights were studied. This second series confirmed apparent stimulatory effects of all levels of PCBs.

There were no differences between treatments in DNA and RNA levels after 24 hours' incubation. The significance of this is not clear, since it had been anticipated that increased RNA levels would accompany growth increases. However, it is thought that differences may have occurred earlier than the 24-hour time.

Data showing uridine uptake by PCB-treated *E. coli* at several phases of growth are presented in Table 3. No significant differences in uptake between treatments and controls were evident except at 5 hours incubation time, when there was a significant diminution of ^3H_U uptake in PCB-dosed bacteria. While not consistent with increased culture yields, the lack of change in uptake between chemically treated *E. coli* and controls does coincide with chemical assays indicating no increased levels of nucleic acids.

Results from these experiments indicate that PCBs stimulate in vitro growth of *E. coli*. If this phenomena occurs in vivo further concern is justified since any environmental contaminant which affects indicator organisms may result in a distorted portrayal of the actual situation.

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Table 2. Lyophilized Weights of *Escherichia coli* Cultures Exposed to PCB (Bacterial Series II).

Treatment	Mean[1] harvest weights (mgm)
PCB .1 μg/ml	186.4
PCB .01 μg/ml	153.2
Control	160.8
Acetone control	133.6
LSD$_{95}$[2]	23.6

[1] Mean of four replicates.

[2] LSD$_{95}$ = Least significant difference at 95% probability level calculated from one way analysis of variance.

Table 3. Tritiated Uridine Uptake by PCB Treated *Escherichia coli* Cultures During Exponential and Decline Growth Phases.

Treatment	Counts per minute[1]		
	5 Hours	8 Hours	24 Hours
PCB .1 μg/ml	4342	2250	2192
PCB .01 μg/ml	4756	2367	2462
Uridine control	8020	2675	2389
LSD$_{95}$[2]	2800	979	859

[1] Mean of four replicates.

[2] Least significant difference at 95% probability level calculated from one way analysis of variance.

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INSECTICIDE TOXICOLOGY

Manifestations of Cottonfield Insecticides In the Mississippi Delta

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A survey of school attendance and mortality records, hospital admissions, and illness among plantation groups was undertaken to determine the possible toxic hazard from the use of modern insecticides in the Mississippi Delta. In all studies reported no evidence could be found that pesticides were the direct or indirect cause of any chronic disease or a contributing cause in diseases generally recognized as having other etiologies. A definite problem exists in relation to widespread use of toxic compounds by inadequately trained and sometimes careless people.

WITH THE INTRODUCTION OF MODERN economic poisons of the chlorinated hydrocarbon and organic phosphorus groups, agricultural practices have undergone considerable revision. The widespread use of these toxic compounds obviously suggests the possibility of acute or chronic intoxication of the resident and particularly of the worker population. It is proper that all physicians should be alert to the possible dangers of new pesticides and that a continuing study of the toxicology of these materials should be made by trained specialists. This point of view should not be confused with the opinion expressed in a few highly publicized reports, both in the popular press (*5*) and in medical literature (*1*, *2*, *4*), which have caused considerable alarm.

The Yazoo-Mississippi Delta is an almost exclusively agricultural area specializing in cotton production. Large quantities of various insecticides are used, primarily for control of cotton insects. During the past 3 years, the Mississippi State Board of Health has been requested repeatedly to determine whether the large-scale use of the newer insecticides presents a toxic hazard to the population of the Delta. This board, being unable to initiate such an investigation at the time, relayed the requests to the Technical Development Branch of the Communicable Disease Center. Because such an investigation had not only local but also general interest, a study was begun in June 1952. Operations were centered at the Cleveland, Miss., Field Station, which is located in the heart of the Delta.

The Yazoo-Mississippi Delta is a flood plain lying between the Yazoo and Mississippi Rivers in northwestern Mississippi. It includes all or almost all of eleven counties and parts of seven more, as shown in Figure 1.

The area is farmed intensively and, because of its physical characteristics, lends itself readily to mechanized cultivation. Many large plantations of several hundred to several thousand acres are located there.

The Delta has been described as "one of the most highly specialized cotton-producing areas in the world" (*6*). This is perhaps not quite as true now as formerly, for some other crops are be-

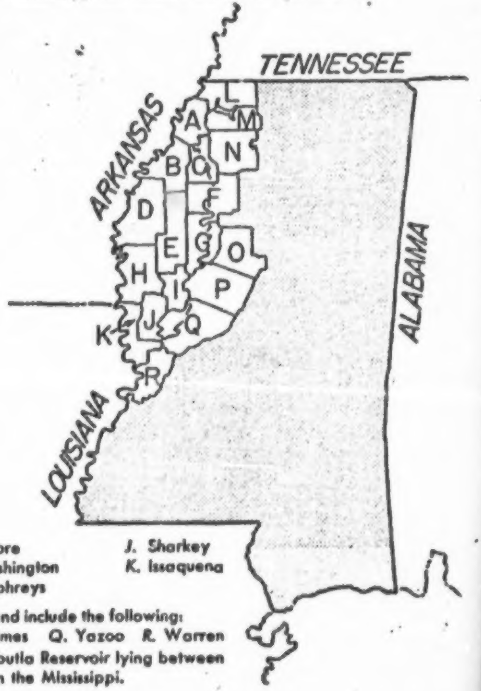

Figure 1. Map of the State of Mississippi showing the Yazoo-Mississippi Delta

Counties lying entirely or almost entirely in the Delta are stippled and are as follows:

A. Tunica	D. Bolivar	G. Leflore	J. Sharkey
B. Coahoma	E. Sunflower	H. Washington	K. Issaquena
C. Quitman	F. Tallahatchie	I. Humphreys	

Counties lying partially in the Delta are not shaded and include the following:
L. DeSoto M. Tate N. Panola O. Carroll P. Holmes Q. Yazoo R. Warren

Headwaters of the Yazoo River pass through Arkabutla Reservoir lying between DeSoto and Tate counties; the river flows south to join the Mississippi.

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coming economically important. However, the Delta still accounts for about half of the cotton produced by the state of Mississippi. Roughly, 50 to 60% of the cropland is in cotton, 12 to 16% in soybeans, 5 to 8% in oats, and 5% in hay. The remainder of the land is occupied by miscellaneous crops, pasture, gardens, and idle cropland. Rice is rapidly assuming a position of importance, and livestock production (beef, hogs, and sheep) has become a major enterprise on a few farms (*3*).

Cotton requires more treatment with insecticides than do the other crops raised in the Delta. Several different compounds are employed for cotton insect control, both as dusts and as sprays, utilizing ground equipment (tractor spray and dust rigs) and air equipment. However, since airplane application of dust and sprays is considered by the local farmers to be less economical and perhaps less effective than ground equipment, it is used only in the latter part of the season when the plants would be damaged by running tractors through them. Favored compounds in the area are aldrin, dieldrin, and toxaphene with the addition of DDT when needed for bollworm control. In addition, benzene hexachloride is used widely in the airplane dusts, frequently in combination with DDT and sulfur in the percentages 3-5-0, 3-10-0, 3-5-40, or 3-10-40 (gamma isomer of BHC-DDT-sulfur). The organic phosphorus insecticides are utilized sparingly, chiefly against infestations of red spiders and aphids which tend to occur in the latter part of July and in August.

Cotton is usually planted beginning the second week in April. Early season insect control is directed against cutworms, thrips, boll weevils, and other minor pests which have survived the winter. Application may be required as soon as the first pair of leaves have spread and may be repeated three or four times at approximately weekly intervals. Mid- and late-season schedules are dependent upon the extent of infestation with the boll weevil and other pests, a 15 to 25% boll infestation demanding three or more treatments at 4- or 5-day intervals. In an average season, approximately 10 applications of insecticide will be required; the range is 6 or 7 to 20 or more applications. Hot dry weather, such as prevailed through the summer of 1952, greatly lessens the amount of poisoning required, because of lessened insect infestation. For example, one large plantation used only about 25,000 pounds of insecticides during the 1952 season as compared to 250,000 pounds during the 1951 season. The poisoning season is usually completed by September 1.

Methods of Investigation

The Mississippi State Board of Health originally requested a study because of recurrent questions from the Delta area regarding the possible effect of the newer pesticides on health. Some of the questions, which are similar to statements published by Biskind (*1*, *2*), were as follows:

1. Does exposure to insecticides bring about a change in intestinal flora leading to gastrointestinal upsets and fungus infections such as thrush?
2. Do insecticides, by their selective action on the central nervous system, cause an increase in cases of insanity?
3. Does insecticide dust suspended in the air cause an increase in hay fever, asthma, and sinusitis?
4. Is there a toxicological hazard from residual DDT on the walls of houses caused by the release of phosgene upon exposure to excessive heat—for example, in winter when heated stoves are in proximity to the walls?
5. Do electrolyte disturbances follow exposure to insecticides? For example, does local gastrointestinal irritation result from exposure and lead to vomiting and thus to dehydration, or does direct stimulation of the respiratory center result from exposure and lead to hyperventilation and thus to alkalosis?
6. Is the increased number of infiltrations noted in films taken by mobile x-ray survey units, and frequently interpreted as virus pneumonitis, actually a result of insecticide exposure?
7. As another stress on the organism, may insecticide exposure contribute to increased incidence of diseases of stress and strain, including neuroses and perhaps even cancer and polio?
8. Through repeated or continuous digestive upsets, does insecticide exposure contribute to malnutrition and deficiency diseases?
9. Do the insecticides cause derangements in enzyme systems and contribute ultimately to chronic diseases such as arteriosclerosis and heart disease?

Because these questions were so broad and so theoretical, it was recognized that obtaining undeniable proof of the absence of a toxicological problem would be nearly, if not quite, impossible, although if such a problem were present to a significant degree, it would not be difficult to obtain proof of the fact. It was felt that the best approach would make use of general morbidity data in several ways, comparing figures when the newer insecticides were in use against a time when they were not in use.

After the general view of medical and agricultural experts in direct contact with the local situation had been obtained, existing statistics were evaluated and morbidity surveys were conducted to study the amount and kind of sickness existing under local plantation conditions. An investigation was made of any reported case of suspected poisoning.

In order to increase the probability of reporting, all physicians were approached through the local medical societies. It was urged that suspected cases of poisoning of whatever nature be reported to the nearest county health officer, or directly to the Cleveland Field Station which was headquarters for the investigation. Other groups, including hospital staffs, county agricultural agents, and civic clubs, were also approached for the same purpose.

Opinion of Local Health Officers and Agricultural Agents

In order to evaluate local feeling concerning the problem of cotton-field insecticide intoxication, several county health officers were first consulted. One reported that the doctors in his county had not complained of the problem to him. Another expressed interest in the possibility of intoxication, but stated that it had not become a problem in his county as judged by his contacts with the physicians. A third stated, in summary, that he had been aware that some laymen and physicians had attributed illness to insecticides, but that he had not found convincing evidence that insecticides play a major role in the causation of illness. Still another stated positively that such a problem did not exist in his county. However, one health officer had received many questions similar to those outlined above and, for this reason, was worried that the problem might be a pressing one. He was anxious that an

Table I. White School Attendance in One Delta County by School Year

School Year	*Total Possible Pupil-Days of Attendance*	*Total Pupil-Days of Absence*	*Absentee Percentage*
A. Before Introduction of Modern Insecticides			
1943–44	713,592	50,041	7.0
1944–45	683,641	45,470	6.7
1945–46	689,927	51,151	7.4
1946–47	705,383	46,202	6.6
Total	2,792,543	192,864	6.91
B. After Introduction of Modern Insecticides			
1947–48	707,584	54,270	7.7
1948–49	760,475	56,776	7.5
1949–50	786,756	57,256	7.3
1950–51	799,280	63,706	8.0
1951–52	801,319	58,528	7.3
Total	3,855,414	290,536	7.53
Grand total	6,647,957	483,400	7.26

investigation be launched to determine the true situation.

As the county agricultural agents are intimately concerned with insecticides and are in close touch with the farmers who use them, an expression of opinion from some of their offices was sought. They felt that the insecticides in current use had proved to be safe, although an occasional case of minor poisoning, usually associated with gross carelessness, had come to their attention.

Review of Existing Statistics

School Attendance Records Total school attendance records for all white children in one Delta county were computed and absentee figures were compared for the 1943–47 period, representing the years before the introduction of the modern agricultural insecticides, and the 1947–52 period, representing the time of widespread use of these compounds (Table I). The absenteeism figures were 6.91 and 7.53%, respectively.

No absolute explanation for the small increase in absence can be offered, although there was an increase in total enrollment during the later period and this increase was derived in part from a group of children who were of school age during the earlier period but did not attend school. When this group did enter school they were a relatively poor risk so far as absenteeism is concerned. Attention is called also to the more general sociological differences between the war and postwar periods, which the figures represent to a large degree. Both rates undoubtedly include absence based on socioeconomic factors as well as illness. A similar set of attendance data for Negro schools of the same county was found to be so incomplete and so inaccurate that it could not be used.

Mortality Reports From the annual statistical summary released by the state of Mississippi, mortality figures were obtained for the state as a whole, and for the following twelve Delta counties: Bolivar, Coahoma, Humphreys, Issaquena, Leflore, Quitman, Sharkey, Sunflower, Tallahatchie, Tate, Tunica, and Washington. Death rates for the Delta and for the state for certain diseases considered germane to this study in view of the previously outlined questions, were compared for 3 years prior to, and 4 years following, the introduction of modern insecticides into this area. These figures are shown in Table II.

Table II. Average Yearly Death Rates per 100,000 Population from Selected Diseases

(For periods 1944–46 and 1947–50 for Delta area and entire state of Mississippi)

	Average Yearly Death Rates per 100,000 Population			
	Delta Area*		Entire State	
Cause of Death	1944–46	1947–50	1944–46	1947–50
Tuberculosis (all forms)	46.3	35.2	40.0	27.7
Dysentery (all forms)	3.9	3.1	2.3	1.8
Diarrhea	10.0	8.0	4.1	5.1
Cancer (including leukemia)	70.1	70.5	78.4	86.2
Heart diseases	141.3	189.4	170.0	213.7
Pneumonia (all forms)	27.4	26.9	34.2	28.0
Crude death rate (all causes)	952.0	921.1	936.7	882.5

* Values given for Delta area represent average for 12 counties.

Death rates decreased in a similar way for both the Delta area and the state as a whole in tuberculosis, dysentery, pneumonia, and deaths from all causes (crude death rate). The Delta showed a decline in death rate for diarrhea, while the state as a whole showed a slight increase. For cancer, the Delta showed no change, while the state as a whole showed an increase. Only in heart disease was there a pronounced rise for the Delta counties, and this was similar to the rise recorded for the state as a whole. Rates for certain diseases, particularly communicable diseases, show a higher figure for the Delta than for the state both before and after the introduction of modern insecticides. This is due in large part to the predominantly white population in other areas of the state as contrasted to the predominantly Negro population in the Delta with the differences in medical care, economic status, and other factors implicit in this distribution.

Hospital Records Records of a hospital located on a large Delta plantation (Plantation A) were reviewed for the period of September 1938 through August 1949. This hospital is operated for the Negro tenant population of the plantation, which averages around 2500 persons.

Records of this hospital furnish a more complete account of illness on the plantation than might be expected, because tenants are hospitalized without cost to themselves and are freely admitted, inasmuch as the company operating the plantation feels it is economically sound to prevent serious illness by means of adequate early care. Furthermore, it is apparent that patients eligible for admission to this hospital are drawn from a population which undergoes a greater exposure to agricultural insecticides than the population from which admissions to most general hospitals are drawn.

Table III. Yearly Amount of Older and Modern Types of Insecticides Purchased for Use on Plantation A for 1945–52

Year	Older Types, Pounds	Modern Types, Pounds
1945	185,000	500 (DDT)
1946	200,000	0
1947	160,000	234,000
1948	0	195,000
1949	119,200	408,000
1950	0	369,800
1951	0	441,500 lb. 11,875 gal.
1952	0	449,950 lb. 19,525 gal.

September 1938 to August 1946 is used as the pre-modern-insecticide period, and September 1946 to August 1949 as the insecticide-exposure period, since 1947 marked the introduction of significant quantities of modern agricultural insecticides on this plantation (Table III). DDT had been used previously in the area as a residual insecticide applied to dwellings. The amount, however, is insignificant when compared to the bulk used for agricultural purposes, and is not considered in this study.

Admission rates for selected diseases are compared in Table IV. The same physician was in charge during the entire period (1938–49) covered by the table. It will be noted that the only disease group showing an increased admission rate in the 1946–49 period is the cardiac group of diseases. This probably reflects the rising trend in cardiac disease shown in all recent studies. All other diagnoses showed a lower rate in the latter period. The total admission rate for September 1951 to August 1952 under a different physician who used different criteria for hospitalization, attempting particularly to bring all maternity cases into the hospital, was 27.2 per hundred population.

Morbidity Surveys

Survey Of Workers This survey was done on Negro male laborers, chiefly tractor drivers, on Plantation A. Brief medical histories were taken and physical examinations were done on 83 individuals who were interviewed afterward by the same medically trained investigator at biweekly intervals from July 9 through September 24, a total of six interviews per man. Some individuals could not be reached every time. At each interview, the laborers were questioned closely and individually about any illness or symptoms occurring since the previous interview and about the type of work done since the last interview. Thus, it was possible at the end of the season to divide the group into an exposure group (occupational contact with insecticides at some time during the season), and a

Table IV. Admission Rates for Selected Diseases at Plantation A Hospital for Negro Tenants

Periods represent eras before and after introduction of modern insecticides

	September 1938–August 1946[a]		September 1946–August 1949[b]	
Diagnosis	*Average annual admissions for specified diagnosis*	*Average annual admission rate per 100*	*Average annual admissions for specified diagnosis*	*Average annual admission rate per 100*
Respiratory diseases	188	6.3	102	4.6
Genitourinary diseases	106	3.6	51	2.3
Gastrointestinal diseases	103	3.3	42	1.9
Cardiac diseases	35	1.2	36	1.6
Rheumatism and arthritis	23	0.8	15	0.7
Nervous disorders	18	0.6	10	0.5
Deficiency diseases	5	0.2	1	..
Metabolic diseases	1	..	1	..
Neoplastic diseases	1	..	3	..
Admissions, all causes	833	28.0	488	22.2

[a] Average annual tenant population 2979. [b] Average annual tenant population 2204.

Table V. Illness Reported in Season-Long Morbidity Survey of 83 Negro Male Farm Laborers on Plantation A

	Group with Occupational Exposure		Control Group	
Symptom or Disease	*No. times occurring*	*Rate[a] per interview week, %*	*No. times occurring*	*Rate[b] per interview week, %*
Headache	18	4.6	16	6.0
Colds	16	4.1	14	5.2
Backache	15	3.9	10	3.7
Skin complaints	10	2.6	..	..
Gastrointestinal complaints	7	1.8	3	1.1
Rheumatism and arthritis	6	1.6	4	1.5
Cardiac complaints	5	1.3	..	..
Infections	4	1.0	4	1.5
Injuries	3	0.8	1	0.4
Hemorrhoids	3	0.8	2	0.8
Allergy	1	0.3	..	..
Toothache	1	0.3	..	..
Miscellaneous	7	1.8	6	2.2
Total morbidity	96	24.9	60	22.4

[a] Based on 386 interview weeks. [b] Based on 268 interview weeks.

control group (no occupational exposure during the interview season); these groups numbered, respectively, 48 and 35 individuals. Information was obtained for 386 interview weeks on the exposure group and 268 interview weeks on the control group. Total illnesses numbered 96 (24.9 per hundred interview weeks) for the exposure group, and 60 (22.4 per hundred interview weeks) for the control group. This is not a statistically significant difference (p = 0.4). These illnesses were of the type expected from any population in a general morbidity survey. Signs and symptoms usually associated with insecticide intoxication were not recognized except as described below. This study is summarized in Table V.

An interesting sidelight observed at the initial examination was the high incidence of elevated blood pressure, usually without associated symptoms. Twenty-seven of the 83 (33%) had pressures above 145 systolic or 90 diastolic. These were found to be distributed as follows: 15 in the exposure group (31% incidence), and 12 in the control group (34% incidence), indicating that the two groups were comparable statistically.

The only diagnosis showing a marked difference in the control and exposure group was the frequency of skin complaints. When the records were reviewed, it was noted that this was accounted for by three individuals who complained repeatedly. One had a chronic fungus infection which had been present since about 1918. The other cases involved irritation by insecticides, and are as follows:

One laborer had a chronically infected ulcerated area on one shin which he irritated by spilling some poison concentrate (probably dieldrin) on it.

A worker spilled a dieldrin concentrate on his left leg and did not wash it off or remove it in any way for several hours. About one week later the skin broke down and blister formations and serious exudation occurred. The skin healed slowly under medical supervision, leaving a depigmented area. The role played by the insecticide and that played by the solvent are uncertain in this case.

Other illnesses which could be related directly to insecticides are as follows:

A tractor driver putting out spray of undetermined type (possibly dieldrin) on a windy day had some blown into his left eye. The eye was treated medically, but remained erythematous and burned for several weeks. Recovery was complete.

A tractor driver putting out both spray and dust of an undetermined composition on a windy day complained of burning in the chest for several days thereafter. Examination of the chest was negative. Recovery was apparently complete.

Survey of General Population

The second type of morbidity survey was carried out at another large Delta plantation (Plantation B). An initial rapid morbidity survey was carried out on the personnel living on this plantation, information being obtained on 639 people. This survey was accomplished during the latter part of July 1952, when the use of insecticides was at a minimum because of a long period of hot, dry weather. The survey data concerned only illnesses for the week prior to the interview in order to hold the memory loss factor to a minimum. Results of this survey are recorded in Table VI.

A similar, follow-up survey was carried out in September after the population had been exposed to atmospheric concentrations of insecticides from airplane dusting of the fields. The experience

Table VI. Results of Morbidity Surveys on Plantation B

	July surveys[a]		September Surveys[b]	
Illnesses within Past Week	*Number*	*%*	*Number*	*%*
Colds and/or sore throats	61	9.6	20	3.4
Headaches	34	5.3	7	1.2
Gastrointestinal complaints	28	4.4	15	2.5
Arthritis and rheumatism	7	1.1	11	1.9
Cardiovascular disease	15	2.3	10	1.7
Infections, including "chills and fever"	7	1.1	5	0.8
Obstetrical and gynecological complaints	7	1.1	5	0.8
Toothaches	6	0.9	2	0.3
Backache	5	0.8	4	0.7
Nervous conditions	4	0.6	3	0.5
Injuries	4	0.6	4	0.8
Asthma	2	0.3	1	0.2
Skin rash	2	0.3	0	0
Miscellaneous	27	4.2	11	1.9
TOTAL	209	32.6	98	16.7

[a] Based on 639 subjects interviewed.
[b] Based on 592 subjects interviewed (3 dead, 44 others not contacted).

of this season was not typical, however, as a total of only about 25,000 pounds of dusts was used as opposed to approximately 250,000 pounds during 1951. This dust, in 1952, was applied to about one third of the cotton by seven applications during August, and that chiefly in the sparsely populated or unpopulated areas. A small amount of defoliant (calcium cyanamide) had also been applied in September to some of the fields which were scheduled for machine picking. Comparisons of the results of this survey with those of the original survey are indicated in Table VI.

Only 592 (93%) of the original 639 individuals were contacted on the follow-up survey. Three of the original group had died, and attempts to reach 44 others were unsuccessful.

Information on the cause of death of the three individuals who died was scanty:

A 36-year old Negro housewife (G.R.) and field worker died suddenly and unexpectedly in July. She had expressed no complaints during the initial interview. The doctor who saw her at the time of death expressed the opinion that she had died of a heart attack.

A 70-year-old Negro man (S.O.) died in August after a prolonged illness. At the time of the initial interview he was under medical care for heart and kidney trouble and had ascites.

A 70-year-old Negro man (G.M.) died in August after a prolonged illness characterized by abdominal pain and emaciation. At the time of the initial interview he was under medical care but no diagnosis had been expressed. Malignancy appears to be the most likely explanation.

The total morbidity was approximately half that of the original survey. This decline in the second survey was accounted for mainly by the respiratory group, headache group, gastroenteritis group, and miscellaneous group of diseases. The reduction in headaches was probably due to cooler weather, since many of the original attacks occurred during the middle of the day in persons doing physical labor when environmental temperatures usually exceeded 100° F. The decrease in gastroenteritis is typical of the seasonal incidence of the disease. An explanation for the decline in the other two groups is not so apparent. Arthritic complaints increased slightly, as could be expected from the changing weather (onset of lower temperatures). Finally, a lower rate would have been obtained in the second survey, even if all the subjects who were not available for questioning at that time had, in fact, been sick.

Reports of Poisoning Cases

It was anticipated that a certain number of cases of acute poisoning would be uncovered because of the widespread use of toxic compounds by a group of inadequately trained and sometimes careless people. The limited number of such cases found is rather surprising in view of the publicity given to the request for complete reporting. In addition to the four cases in the Plantation A survey, the following cases were discovered through other sources:

One case of acute mild aldrin poisoning occurred in a Negro tractor driver who tried to clean clogged spray nozzles by blowing through them. His face, arms, and legs were thoroughly saturated, and he probably inhaled and swallowed small amounts in addition. Shortly thereafter he developed headache, nausea, burning of the skin, and jitteriness. He was treated with phenobarbital and recovered completely within 48 hours.

Two cases of nausea, vomiting, and weakness occurred in a house where window fans were operating while a plane was dusting a field directly behind the house with 1% parathion dust. Onset of symptoms occurred within 30 minutes of the time the plane began putting out dust. One of these individuals appeared completely recovered within 6 hours, but the other continued to complain of weakness for several days.

No other cases which seemed to have any reasonable connection with insecticides were reported.

Summary and Conclusions

A survey of local expert opinion in the Yazoo-Mississippi Delta area showed that local county agricultural agents who were in close touch with farmers and four county health officers who were in close communication with physicians did not consider that there was any evidence in the general population of widespread chronic intoxication by insecticides. A fifth county health officer felt that the problem might be a pressing one and should be investigated. The county agents were aware of isolated instances of minor poisoning.

A comparison of the total school attendance records for all white children in one Delta county for the 1943–47 period, representing the years before the introduction of the modern agricultural insecticides, with those for the 1947–52 period, representing the time of widespread use of these compounds, showed that the absentee figures were slightly higher for the latter period (7.53%) than for the former (6.91%).

Mortality figures for 1944–46 and for 1947–50 were obtained for the entire state of Mississippi, and an average mortality rate was calculated for twelve of the Delta counties for the same periods. Of the seven causes of death studied, only heart disease showed a pronounced rise for the Delta counties during the modern-insecticide era, as compared with the earlier period, and this was similar to the rise recorded for the state as a whole. These mortality figures indicated a general improvement in health conditions in the Mississippi Delta as well as the entire state.

Morbidity records for a Delta plantation hospital showed a lower rate for all diagnoses except cardiac diseases in the 1946–49 period than in the 1938–46 period.

A morbidity survey on 48 exposed and 35 unexposed laborers showed no marked difference between the two groups except in the frequency of skin complaints. Part of these complaints were caused by fungus disease and part by direct, excessive exposure to insecticides.

A morbidity survey on 639 unselected people living on a large plantation was carried out in July when the use of insecticides was still at a minimum, and was repeated in September after the population had been exposed to atmospheric concentrations of insecticides from airplane dusting of the fields. The total morbidity in September was approximately half that of the original survey.

Seven cases of acute insecticide poisoning were found in the Delta area during the summer of 1952.

No one of the studies reported above can be considered absolutely conclusive. There are variables other than insecticide usage which cannot be ruled out as contributing factors in these studies, especially in the comparison of morbidity or mortality rates for two different chronological periods. However, in all the studies reported, no evidence could be found that pesticides were the direct or indirect cause of any chronic disease, nor a contributing cause in diseases generally recognized as having other etiologies.

A definite, though not extensive, problem exists in relationship to the widespread use of toxic compounds by a class of inadequately trained and sometimes careless people. This problem is in the nature of acute, often mild, intoxication due to excessive exposure which is frequently associated with gross carelessness. Improvement in this aspect may be expected on a long-term basis in view of the intensive and extensive educational program being carried on by physicians, county agricultural agents, agents of insecticide-producing companies, agricultural experiment station staffs, and other interested people.

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DILATATION OF THE PUPILS IN PARATHION POISONING

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With the advent of the organic phosphorus insecticides, it has become important for all physicians who may be called upon to serve persons in agricultural pursuits to familiarize themselves with the salient features of the high inherent toxicity of these cholinesterase-inhibiting compounds and the special treatment required for poisoned individuals. A review prepared by the Committee on Pesticides of the American Medical Association contains much information on the toxicology and pharmacology of these insecticides and on the diagnosis and treatment of human poisoning by them.[1]

A generally known and accepted sign of poisoning from the standpoint of establishing an early diagnosis has been that of extreme miosis (pin-point pupils), which develops after the inhibition of cholinesterase and as a consequence of marked parasympathomimetic stimulation of the iris. Treatment, which is directed at counteracting the cholinergic action with large doses of atropine, is usually guided, in part at least, by the degree of mydriasis produced, although control of more significant but less readily observed functions should be attained.[2]

A number of reports have indicated that miosis is not a constant finding in animals[3] or man[4] after sufficient exposure to a number of organic phosphorus compounds to produce systemic effects. However, in view of the emphasis that has been accorded the degree of pupillary constriction as a finding essential to the diagnosis and as a gauge of the adequacy of therapy, the two recent cases that follow appear worthy of record. They represent instances of severe illness due to poisoning from the organic phosphorus insecticide, parathion, in which the failure of miosis to occur early in the illness resulted in delay in diagnosis and, therefore, in delay in the institution of adequate treatment.

Report of Cases

Case 1.—A 15-year-old boy had been engaged in the loading of a 45% solution of parathion into airplanes (3 gal. and 1 pt. of emulsifiable concentrate with water to make 50 gal.) and in flagging the course of the plane during spraying for a period of two weeks. He experienced frequent, small respiratory exposures to the 3.5% spray and on several occasions got small amounts of the concentrated stock solution on his skin. His only unusual exposure occurred on July 11, 1953, at 5:30 p. m., when, in loading a plane, he spilled a small amount of the parathion concentrate on his skin. Until that time he had felt well in all respects, but within an hour he developed nausea, vomiting, headache, diaphoresis, confusion, and awkward gait. On examination at 6:30 p. m. it was observed that his pupils were dilated, the pulse was rapid, and the reflexes were hyperactive. In view of the extreme heat of the day and the dilatation of the pupils, the physician felt that the patient was suffering from heat exhaustion; however, as the patient had been exposed to parathion, he did empirically administer 0.6 mg. of atropine sulfate hypodermically. The patient was put to bed and soon was asleep. At 8:30 p. m. he awakened with muscular twitching, ptyalism (sialorrhea), and diaphoresis and was observed to have developed extreme miosis. It was then that the true nature of the illness became apparent, and appropriate steps were taken to remove him to a hospital. Atropine, 0.6 mg., was administered again at 8:45 p. m., and a third dose was given just before the patient was placed in the ambulance at 9:15 p. m. The patient was taken over 100 miles to a hospital. On arrival (1:30 a. m., July 12, 1953) he was critically ill, being comatose, in opisthotonos, having repeated clonic convulsions, and exhibiting other manifestations of severe poisoning. Intensive therapy consisted of frequent doses of atropine and supportive measures. In addition to the three doses received for first aid, the dose of atropine in the hospital was 0.6 mg. given approximately every 20 minutes for 8 doses, then 0.4 mg. every hour for 8 doses, then every three hours as needed for 5 doses, the total dose being 11.6 mg. Other treatment included introduction of an indwelling catheter and administration of phenobarbital (Luminal) sodium, and 5% glucose solution. The first improvement was noted at 4 a. m., and the pupils began dilating at 4:30 a. m. By 8 a. m., at which time the pupils became fully dilated, he was considered out of danger. Eventual recovery was complete. The diagnosis was confirmed by the finding of extremely low levels of cholinesterase activity. At 10:30 a. m. on July 12, 1953, cholinesterase activity of the erythrocytes was 0.1 Δ pH per hour and of the plasma, 0.08 Δ pH per hour by the Michel technique.[5]

Case 2.—A 3-year-old girl, who had been in excellent health, on July 19, 1953, at 1:30 p. m. spilled an unknown quantity of 25% solution of parathion on her thigh from a container that had been carelessly left within her reach. A few minutes later the mother bathed her and put her to bed. Upon awakening at 3 p. m. she was restless and irritable and had difficulty in walking. She complained of headache and gradually became more ill. A physician was called, and at 4:30 p. m. he observed that she was unsteady on her feet, had difficulty holding her head erect, had dilated pupils, and appeared ill. He was shown the container, whereupon he recognized the possibility of parathion poisoning and, despite being perplexed by the dilated pupils, administered 0.5 mg. of atropine orally. She was taken to the hospital and on arrival at 5:30 p. m. was unresponsive. In the interim she had developed pulmonary edema, hyperactive reflexes, fine muscular tremors, elevated blood pressure, ptyalism, and other manifestations of serious illness, but the pupils had remained large. She was given 0.3 mg. of atropine parenterally at 5:40 p. m. As the pupils were not constricted, although she remained seriously ill, no additional atropine was given until 7 p. m., at which time moderate miosis had developed despite the dosage of atropine. Subsequent full atropinization evoked prompt improvement, and

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full recovery followed. Extremely low levels of blood cholinesterase activity again confirmed the diagnosis. At 6 p. m. on July 19, 1953, cholinesterase activity of the erythrocytes was 0.06 Δ pH per hour and of the plasma, 0.04 Δ pH per hour by the Michel technique.

Comment

In view of the apprehension that obviously attends the onset of such forceful symptoms as those produced by organic phosphorus insecticide poisoning, it is not unrealistic to consider that pupillary dilatation might occur, especially initially, as the result of excessive stimulation of the sympathetic nervous system.[4b] Certainly fear, anger, pain, and some other emotional stimuli are known to cause dilatation of the pupil. Regardless of the mechanism, the size of the pupil is subject to numerous influences, and it would appear unwise to regard pupillary status alone as essential to diagnosis or as important for measuring the success of treatment of parathion poisoning.

The first case points out the striking similarity between the clinical patterns that may be observed in heat exhaustion and in parathion poisoning. Since the etiological circumstances of each of these conditions are frequently encountered concurrently in spraying operations during hot weather, it is necessary that they be differentiated in establishing the diagnosis.

Summary

In two severe but nonfatal cases of poisoning from dermally absorbed parathion, initial dilatation of the pupils resulted in delay in accurate diagnosis and adequate treatment, especially full atropinization. Overemphasis was accorded miosis. There is need for reliance on a carefully taken history of exposure and on a comprehensive analysis of all signs and symptoms. The presence or absence of miosis is of practical but limited significance in the differential diagnosis of early parathion poisoning.

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DERMAL ABSORPTION OF HEXACHLOROPHANE IN INFANTS

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Summary Hexachlorophane is widely used as an antibacterial agent. It has been reported that repeated high oral doses of hexachlorophane (25 mg. per kg. per day) in rats produced paralysis and a pronounced œdema of the white-matter of the central nervous system. To see whether hexachlorophane may be absorbed through the intact infant skin, its concentration in the blood of the umbilical cord of fifty infants and in the blood of the same infants obtained on discharge from hospital was determined by gas chromatography. While in the hospital the infants were washed several times with hexachlorophane solutions to prevent staphylococcal infections. The range of hexachlorophane levels in the umbilical cord was 0·003–0·182 p.p.m. (μg. per g.), with a mean of 0·022 p.p.m., and for the blood at the time of discharge from the hospital 0·009–0·646 p.p.m., with a mean of 0·109 p.p.m. It is suggested that infants' skin be rinsed after the use of hexachlorophane.

INTRODUCTION

HEXACHLOROPHANE [2,2′-methylene-bis-(3,4,6-trichlorophenol)] is used extensively as an antibacterial agent. It is incorporated in soaps, cosmetics, and antiseptic solutions. Kimbrough and Gaines have found that high doses (25 mg. per kg. per day) of hexachlorophane given for 2 weeks in the diet of adult rats was followed by paralysis of their hindlegs.[1] After a single dose of 100 mg. per kg. hexachlorophane given by stomach tube, weaning rats became paralysed.[2] Histological examination of the rat brains revealed striking vacuolisation of the white-matter. Electron-microscopy disclosed that the large vacuoles in the white-matter were lined by portions of myelin sheaths.

The vacuoles contained very little electron-dense material or seemed to be completely empty. These lesions were interpreted as œdema of the cerebral white-matter, and resembled the diffuse spongy degeneration of the white-matter of the brain described in infants.[3] They seemed identical with the lesions produced in animal brains with triethyl-tin.[4]

A review of the toxicity of hexachlorophane[5] suggested that this compound when applied dermally might be absorbed through intact skin. This was substantiated in animal experiments and by analysis of human blood from fourteen adults with no unusual exposure to this compound.[6]

Washing of newborn infants with hexachlorophane emulsions is advocated to reduce staphylococcal colonisation and infections in hospital nurseries.[7] It is also recommended that thorough rinsing be avoided to allow a film of hexachlorophane detergent solution to remain on the infant's skin.

To test for absorption of this substance through the intact skin, hexachlorophane concentrations were determined in the umbilical cord blood of fifty healthy newborn babies at birth and in their blood after several washings.

METHODS

Fifty newborn infants at a single hospital during the late autumn of 1970 comprised the study population. The established nursery routine specified daily washings with hexachlorophane following the technique of Gluck and Wood.[7] No new instructions were issued during the study period. The hexachlorophane washes had been in use for 2 years after a staphylococcal outbreak in the nursery.

At birth 1·5 ml. of cord blood was collected in an oxalated tube. A second sample of 1·5 ml. of blood was collected in an oxalated tube from a peripheral vein of each infant on the day of its discharge from the hospital. During the hospital stay, 1–11 days, the infants were washed once daily with a diluted 3% hexachlorophane detergent solution. The diluent varied from 50 to 100 ml. of warm water. At times, the hexachlorophane solution was gently rinsed off and at other times it was left on the skin according to the interpretation of the physicians' instructions by the individual nurses. The amount of the applied commercial hexachlorophane-containing detergent solution averaged approximately 3·5 g. The hexachlorophane applied was thus slightly over 100 mg. Except for seven cases, accurate records of the washings were available in the infants studied.

1 ml. of blood was taken from each sample and extracted

with 4 ml. of ether. The method described in detail by Curley and Hawk[8] was used for the determination of hexachlorophane.

The extracts were methylated with diazomethane in ether. The diazomethane was prepared from N-methyl-N′-nitro-N-nitrosoguanidine by the method of Stanley.[9] The extracts were allowed to react at least 4 hours. The methylated extracts were evaporated to dryness at 35°C and purified on silica-gel microcolumns.

Electron-capture gas-liquid chromatography ('Varian 2100') was done on a ¼ in. × 16 in. column of 3% diethyleneglycol succinate/2% phosphoric-acid on 30/60 acid-washed 'Chromosorb W'. The column and injector temperatures were 190°C and 215°C, respectively. The carrier-gas (nitrogen) flow-rate was 50 ml. per minute. The retention-time of the derivative was approximately 4 minutes. The minimal detectable amount of the methylated derivative was 0·04 ng. or less, depending on the settings of the instrument. The results were expressed as µg. hexachlorophane per g. whole blood (p.p.m.).

RESULTS

The concentrations of hexachlorophane for whole cord blood ranged from 0·003 p.p.m. to 0·182 p.p.m., with a mean of 0·022 p.p.m., standard error ±0·005. For whole blood taken at time of infant's discharge from hospital, concentrations ranged from 0·009 p.p.m. to 0·646 p.p.m., with a mean of 0·109 p.p.m., standard error ±0·016. In most cases the hexachlorophane concentration found in the blood of the infants was higher on the day of discharge from hospital than at birth. The highest concentration (0·646 p.p.m.) was in a baby boy after five washings. After fourteen washings the concentration of hexachlorophane in another infant was 0·250 p.p.m. The concentration of hexachlorophane in five infants was higher at birth than it was at the time of their discharge. In one infant, hexachlorophane was not detected in the cord blood, but was present in the discharge blood. Neither weight nor sex seemed to influence the hexachlorophane blood-levels.

DISCUSSION

Any hexachlorophane found in umbilical cord blood, assuming it was not a contaminant during delivery, would have had to pass through the placenta. In an earlier study,[8] hexachlorophane blood-levels were determined in fourteen blood-samples from adults with no unusual exposure to the compound. These blood concentrations ranged from <0·001 to 0·089 p.p.m., with a mean of 0·028, S.E.M. ±0·008 p.p.m. Three cord-blood samples had concentrations higher than that of the highest adult concentration. The use of hexachlorophane preparations by the mother (e.g., in a vaginal spray) may have contributed to the higher blood-levels. In addition, a " prep " solution containing hexachlorophane was used on every woman varying from several hours before to immediately before delivery. Large variations in the concentration of hexachlorophane in the blood were found at the time of discharge of the infants from the hospital. This fact could not be correlated with the number of washings. Two factors might have influenced these concentrations. Probably, less hexachlorophane was absorbed when the material was rinsed off after washing, but we do not know which babies were rinsed or how thoroughly rinsing was carried out. The interval between the last washing and the time when the blood-sample was taken could possibly influence the concentration of hexachlorophane in the blood.

After oral ingestion in animals hexachlorophane is metabolised in the body, and excreted in the urine and to a greater extent in the fæces. Maximum excretion is reported to occur during the first 72 hours.[9] When a dose of 10 mg. per kg. of hexachlorophane 3% detergent solution was applied to intact rabbit skin, the highest mean blood-level (0·424 p.p.m.) was reached in 7 hours. After 48 hours, the mean concentration in blood was 0·074 p.p.m.[10] The work of Shemano and Nickerson [11] indicates that hexachlorophane accumulates on the skin even though gentle rinsing for 30 seconds is practised. Whether hexachlorophane is absorbed or metabolised as rapidly in man as it is in laboratory animals is not known.

The dietary intake of 100 p.p.m. (5 mg. per kg. per day) hexachlorophane in rats for 97 days or longer slightly affected the brains of some rats and slightly reduced the survival rate of offspring of the F_1 but not the F_2 generation.[8] The concentration of hexachlorophane in the blood of these rats ranged from 0·985 to 1·48 p.p.m., with a mean of 1·21 p.p.m., S.E.M. ±0·17 p.p.m. These concentrations were not reached when infants were washed with hexachlorophane-containing solutions. The highest level obtained in the infants (0·646 p.p.m.) was about two-thirds that of the lowest concentration of hexachlorophane in the blood of rats showing a slight toxic effect.

Our findings indicate that hexachlorophane is present in the umbilical cord blood of infants at birth, implying, in the absence of direct contamination during delivery, a placental transfer. After birth when hexachlorophane has been applied to the skin the blood concentration generally increases. Some of it may be ingested if the material is left on the babies' hands, but most of it is absorbed through the skin. Thus far, no toxicity has been described in infants washed in this way. Convulsions in an infant 4 days after repeated applications of the 3% emulsion to the skin without subsequent rinsing have been reported.[12]

These data raise questions about the safety of hexachlorophane when it is allowed to accumulate on the skin without rinsing and suggest that the technique of washing infants with hexachlorophane be re-evaluated. It would seem advisable to rinse the skin carefully after the application of hexachlorophane.

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